(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 August 2002 (22.08.2002)

PCT

(10) International Publication Number
WO 02/064804 A2

(51) International Patent Classification[7]: C12N 15/82, 5/14, A01H 5/00, 5/10

(21) International Application Number: PCT/US02/04188

(22) International Filing Date: 13 February 2002 (13.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/268,358 13 February 2001 (13.02.2001) US

(71) Applicant: **UNIVERSITY OF FLORIDA** [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US).

(72) Inventors: **LI, Zhijian**; 2807 Binon Road, Apopka, FL 32703 (US). **GRAY, Dennis, J.**; 24734 Turkey Lake Road, Howey-in-the Hills, FL 34737 (US).

(74) Agents: **SAMPLES, Kenneth, H.** et al.; Fitch, Even, Tabin & Flannery, 120 South LaSalle Street, Suite 1600, Chicago, IL 60603 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/064804 A2

(54) Title: A BI-DIRECTIONAL DUAL PROMOTER COMPLEX WITH ENHANCED PROMOTER ACTIVITY FOR TRANSGENE EXPRESSION IN EUKARYOTES

(57) Abstract: The present invention is directed to bidirectional promoter complexes that are effective for enhancing transcriptional activity of transgenes. The bidirectional promoters of the invention include a modified enhancer region with at least two core promoters on either side of the modified enhancer in a divergent orientation.

A BI-DIRECTIONAL DUAL PROMOTER COMPLEX WITH ENHANCED PROMOTER ACTIVITY FOR TRANSGENE EXPRESSION IN EUKARYOTES

The present application is a non-provisional application claiming priority under 35 USC 119(e) to U.S. Provisional Application No. 60/268,358, of Li et al., entitiled A BI-DIRECTIONAL DUAL PROMOTER COMPLEX WITH ENHANCED PROMOTER ACTIVITY FOR TRANSGENE EXPRESSION IN EUKARYOTES, filed February 13, 2001, which is incorporated herein in its entirety by reference.

The present invention relates to bidirectional dual promoter complexes (BDPC) for enhancement of transgene expression. More particularly, a BDPC is constructed by placing two core promoters on either side of modified enhancers.

BACKGROUND

Gene expression is composed of several major processes, including transcription, translation and protein processing. Among these processes, transcription not only dictates the precise copying of DNA into mRNA but also provides sophisticated mechanisms for the control of gene expression. There are a number of fundamental steps involved in transcription: promoter recognition and binding by transcription factors and RNA polymerase components, nascent RNA chain initiation, RNA transcript elongation, and RNA transcript termination (Uptain et al., Ann. Rev. Biochem. 66:117-172 (1997)). Promoters are an essential component for transcription, effecting transcription both quantitatively and qualitatively. A promoter contains numerous DNA motifs or cis-elements that can serve as recognition signals and binding sites for transcription factors. Working

together with transcription factors, these cis-elements can function as architectural elements or anchoring points for achieving promoter geometry (Perez-Martin et al., Ann. Rev. Microbiol. 51:593-628 (1997)).

Numerous promoters have been isolated from a wide variety of organisms ranging from viruses to animals. They have become the subjects of intensive studies in efforts to characterize their molecular organization and the basic mechanisms regulating transcriptional control of gene expression. In recent years, a number of well-characterized promoters have been successfully adopted for use in the genetic transformation of plants. These promoters control transgene expression in transgenic plants and have been used in efforts to improve agronomic performance and to incorporate value-added features. However, in spite of the availability of these promoters, there is currently a shortage of promoters for use in genetic transformation research with plants. In most instances, use of existing plant promoters isolated from a specific species to effect transformation in a different species results in reduced promoter activity and/or altered patterns of gene expression, reflecting the variation of genetic background between different species (Ellis et al., EMBO J. 6:11-16 (1987); Miao et al., Plant Cell 3:11-22 (1991)). Recently, a constitutive actin gene promoter isolated from *Arabidopsis* (An et al., Plant J. 10:107-121 (1996)) failed to support desired levels of transgene expression in grape cells. To date, the promoter most commonly used to effect transformation in crop plants is the cauliflower mosaic virus 35S (CaMV 35S) promoter and its derivatives (Sanfacon, Can. J. Bot. 70:885-899 (1992)). The CaMV 35S promoter was originally isolated from a plant virus.

Successful genetic transformation of plants frequently requires the use of more than one promoter to

adequately drive expression of multiple transgenes. For instance, at least three promoters are normally needed in order to express a selectable marker gene, a reporter marker gene and a target gene of interest. Multiple promoters are required because almost all the mRNAs in eukaryotes are monocistronic (single polypeptide-encoding transcript). Hence, expression of complex traits controlled by more than a single target gene in plants has been thought to require the use of additional promoters.

Recent studies have showed that foreign DNA integrated into the plant genome can be recognized by host factors and that the foreign DNA may be subsequently subjected to modifications that lead to transgene silencing. Mechanisms involved in this process include; DNA methylation, chromatin structural modification and post-transcriptional mRNA degradation (Kumpatla et al., TIBS 3:97-104 (1998)). In general, foreign DNA containing repeated sequences, including sequences homologous to host DNA, is more prone to gene silencing modifications (Selker, Cell 97:157-160 (1999)). Accordingly, the repeated use of the same promoter in transformation vector may increase the probability of gene silencing and unstable transgene expression in transgenic plants. As more transgenic crop plants are developed for release to the farmers, transgene silencing is likely to become a major concern. Hence, there is an urgent need to develop new promoters that will efficiently drive transgene expression, especially in transgenic plants.

Over the years, several strategies have been adopted for use to improve the performance of various promoters. These strategies can be classified into two categories, namely 1) modification of homologous promoters and 2) construction of heterologous promoters.

Modification of homologous promoters is accomplished by manipulating the enhancer region of a particular promoter in an effort to achieve higher transcriptional activity without altering existing expression patterns. Kay et al. (Science 236:1299-1302 (1987) first demonstrated that approximately ten-fold higher transcriptional activity was achieved by tandem duplication of 250 base pairs of the upstream enhancer region of the CaMV 35S promoter, as compared to the transcriptional activity of the natural promoter. Mitsuhara et al. (Plant Cell Physiol. 37:49-59 (1996)) further showed that other forms of tandem repeats of the upstream enhancer region of the CaMV 35S promoter were also capable of producing 10 to 50 fold higher levels of transgene expression in rice and tobacco without altering the constitutive expression pattern of the promoter.

Modification of promoters using heterologous enhancer sequences is also commonly practiced to achieve higher transcriptional activity and desired expression patterns. For example, a CaMV 35S promoter upstream enhancer fragment was fused to the nopaline synthase promoter (NOS) and the resulting fusion promoter reportedly increased the transcriptional activity, as compared to the weaker NOS promoter (Odell, et al. PMB 10:263-272 (1988)). The upstream enhancer regions of the CaMV 35S promoter and the octopine synthase promoter were used to fuse with the maize Adhl promoter to enhance transcription activity, while retaining the anaerobic regulation pattern of the Adhl promoter (Ellis et al. EMBO J.6:11-16 (1987) and 6:3203-3208 (1987)). The achievement of transcriptional enhancement by using heterologous enhancers is primarily attributable to the unique characteristics of enhancers, which could exert its functions to regulate transcriptional activity in an orientation- and position-independent fashion.

SUMMARY

The present invention is directed to a bidirectional dual promoter complex (BDPC) for enhancement of transgene expression and a method for constructing a BDPC. In accordance with the invention, the BDPC includes at least two core promoters and at least one modified internal enhancer region. The core promoters are fused to either end of the modified enhancer region in a divergent orientation such that the transcriptional direction (5' to 3') of each promoter points away from each other (see for example Fig. 1). The modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity. Each core promoter is capable of independently directing transcription of a transgene that may contain expressible or nonexpressible coding sequences.

In another aspect of the invention, both enhancer and core promoter components used in a BDPC may be derived from homologous and/or heterologous promoter sequences. More specifically, in a homologous BDPC, the repeated enhancer sequences and core promoters may be isolated from a single source promoter that is composed of an enhancer and a core promoter. In a heterologous BDPC, the repeated enhancer sequences may be isolated from a promoter source that is different from that which the source promoter from which the core promoters are obtained.

The core promoter of the present invention includes a DNA sequence that corresponds to about 50 bp to about 100 bp. The core promoter may include a TATA-box consensus element and an Initiator (INR). In another aspect of the invention, the core promoter includes a TATA-box consensus element, an INR, and at least one cis-acting element such as a CAAT-box or an as-1 element (Benfey et al., Science 250:959-966 (1990)). Core promoters in a BDPC may have substantial sequence

identity or in one aspect of the invention, be identical. In another aspect, the core promoters of the invention may have a sequence homology of at least about 30% and include at least 5 bp identical, contiguous nucleotides within the core promoter region.

The modified enhancer region in the BDPC may include at least two enhancer sequences having substantial sequence identity arranged in a tandem orientation. In one aspect, the enhancer sequences are identical. The modified enhancer regions are constructed such that the 3' end of a first enhancer sequence is linked to the 5' end of a second enhancer sequence to form a modified enhancer region of the BDPC of the invention. In another aspect, more than two, or multiples of two, such as four and six, repeated enhancer sequences can also be used to construct a BDPC. In an aspect of the invention where four enhancer sequences are used, a first tandem two-unit enhancer region may be fused with another tandem two-unit enhancer region in a back-to-back orientation. The DNA sequence of each enhancer region in a BDPC may be about 100 bp to about 1.0 kbp. In one aspect, transcriptional efficiency is increased when enhancer regions are asymmetrical. The size of an enhancer region is based on desired requirements for the level of transcriptional activity and on desired requirements for a specific transgene expression regulation mechanism.

The modified enhancer region of the BDPC of the invention may also include enhancer sequences that are fully functional to the core promoters used in the BDPC. In this aspect of the invention, enhancers that are fully functional are capable of modulating, including enhancing or down regulating, the initiation and synthesis of transcripts from a transgene containing either translatable or non-translatable coding sequences.

In another aspect, the BDPC of the invention is utilized to provide simultaneous control of transgene

transcription and expression from both core promoters whose transcriptional activities are significantly enhanced by the arrangement of the promoter complex. The use of the BDPC of the invention in transgenic hosts is effective for providing enhanced levels of transcription in both transient expression and stable transformation assays. In this aspect of the invention, by using a homologous BDPC that includes two modified enhancer regions and two core promoters, all of which are derived from the same source promoter, up to a 220-fold increase in transcriptional activity was obtained from an upstream core promoter as compared to transcriptional activity from the same core promoter alone (see Fig. 13). Up to a 2-fold increase in transcription activity can be achieved from an upstream core promoter in a BDPC as compared to that same core promoter having the same enhancer sequences but not in a BDPC. Further, transcriptional activity may be increased as much as 40% in a downstream core promoter in a BDPC as compared to a double enhancer with a core promoter.

In another aspect, the present invention is effective for increasing the number of transcription units and for enhancing transcription control based on the use of a limited number of promoter sequences. Since DNA sequences from a single promoter source can be used to construct a homologous BDPC for the expression of two, or more than two in the case of translation fusion, monocistronic transgene sequences, the number of promoters required to express multiple transgenes is reduced by using the BDPC of the invention. In addition, expression of these multiple transgenes is under the control of the same BDPC and regulated simultaneously according to regulatory information encoded within the shared enhancer region and core promoters. Accordingly, the BDPC of the present invention is effective for achieving synchronized expression of complex multi-gene-

controlled quantitative traits loci (QTL), including those responsible for major events of growth and development in crop plants and other higher organisms. In this aspect, the invention provides transgenic plants, asexual cuttings from these plants in certain instances, and seeds from transgenic plants in certain instances, that contain the BDPC of the present invention. The BDPC of the present invention are also effective for reducing transcriptional silencing of transgene expression.

Examples of BDPCs are set forth in Figure 2 (SEQ. ID. Nos.: 1 and 2), Figure 4 (SEQ. ID. Nos.: 3 and 4), Figure 6 (SEQ. ID. Nos.: 5 and 6), Figure 8 (SEQ. ID. No.: 7 and 8), Figure 10 (SEQ. ID. No.: 9 and 10) Figure 12 (SEQ. ID. No.: 11 and 12), Figure 19 (SEQ. ID. No.: 13 and 14), Figure 21 (SEQ. ID. No.: 15 and 16), and Figure 23 (SEQ. ID. No.: 17 and 18).

BRIEF DESCRIPTION OF FIGURES

Figure 1 illustrates a BDPC with 2 enhancers based on CaMV 35S promoter.

Figure 2 shows the nucleotide sequence (SEQ. ID. Nos.: 1 and 2) of the BDPC illustrated in Figure 1.

Figure 3 illustrates a BDPC with 4 enhancers based on CaMV 35S promoter.

Figure 4 shows the nucleotide sequence (SEQ. ID. Nos.: 3 and 4) of the BDPC illustrated in Figure 3.

Figure 5 illustrates a BDPC with 2 enhancers based on CsVMV promoter.

Figure 6 shows the nucleotide sequence (SEQ. ID. Nos.: 5 and 6) of the BDPC illustrated in Figure 5.

Figure 7 illustrates a BDPC with 4 enhancers based on CsVMV promoter.

Figure 8 shows the nucleotide sequence (SEQ. ID. Nos.: 7 and 8) of the BDPC illustrated in Figure 7.

Figure 9 illustrates a BDPC with 2 enhancers based on ACT2 promoter.

Figure 10 shows the nucleotide sequence (SEQ. ID. Nos.: 9 and 10) of the BDPC illustrated in Figure 9.

Figure 11 illustrates a BDPC with 2 enhancers based on PRb1b promoter of tobacco.

Figure 12 shows the nucleotide sequence (SEQ. ID. Nos.: 11 and 12) of the BDPC illustrated in Figure 11.

Figure 13 illustrates a physical map of the T-DNA region of binary vectors containing a BDPC.

Figure 14 illustrates transient GFP expression in grape SE (somatice embryo, *Vitis vinifera* cv. Thompson Seedless) after transformation using binary vectors p201 and p201R.

Figure 15 shows transient GFP expression efficiency of grape SE (*Vitis vinifera* cv. Thompson Seedless) after transformation using binary vectors p201 and p201R.

Figure 16 shows an analysis of GUS activity in grape SE (*Vitis vinifera* cv. Thompson Seedless) after transformation using binary vectors p201 and p201R.

Figure 17 illustrates GFP expression in grape SE(A) and leaf tissue (B) of transgenic grape (*Vitis vinifera* cv. Thompson Seedless) containing the T-DNA of p201R.

Figure 18 illustrates a BDPC with 2 enhancers based on At UBQ1 promoter.

Figure 19 shows the nucleotide sequence (SEQ. ID. Nos.: 13 and 14) of the BDPC illustrated in Figure 18.

Figure 20 illustrates a heterologous BDPC with 2 UBQ-1 enhancers and 2 CsVMV core promoters.

Figure 21 shows the nucleotide sequence (SEQ. ID. Nos.: 15 and 16) of the BDPC illustrated in Figure 20.

Figure 22 illustrates a heterologous BDPC with 2 PR1b enhancers and 2 CaMV 35S core promoters.

Figure 23 shows the nucleotide sequence (SEQ. ID. Nos.: 17 and 18) of the BDPC illustrated in Figure 22.

Figure 24 illustrates a physical map of a T-DNA region of CaMV 35S promoter-derived binary vectors containing a BDPC.

Figure 25 shows the analysis of GUS activity in three different grape SE (*V. Vinifera cv.* Thompson Seedless) lines after transformation using three binary vectors.

Figure 26 illustrates a physical map of a T-DNA region of transformation vectors with 4-enhancer-containing BDPC.

Figure 27 shows the analysis of GUS activity in SE (*V. Vinifera cv.* Thompson Seedless) lines after transformation using three binary vectors.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al. (1994) Dictionary of Microbiology and Molecular Biology, second edition, John Wiley and Sons (New York) provides one of skill with a general dictionary of many of the terms used in this invention. All patents and publications referred to herein are incorporated by reference herein. For purposes of the present invention, the following terms are defined below.

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, or sense or anti-sense, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence includes the complementary sequence thereof.

The terms "operably linked", "in operable combination", and "in operable order" refer to functional linkage between a nucleic acid expression control sequence (such as a promoter, signal sequence, or array

of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence affects transcription and/or translation of the nucleic acid corresponding to the second sequence. In the present application, the gene of interest that is operably linked to the BDPC may be upstream or downstream from the BDPC.

The term "recombinant" when used with reference to a cell indicates that the cell replicates a heterologous nucleic acid, expresses said nucleic acid or expresses a peptide, heterologous peptide, or protein encoded by a heterologous nucleic acid. Recombinant cells can express genes that are not found within the native (non-recombinant) form of the cell. Recombinant cells can also express genes that are found in the native form of the cell, but wherein the genes are modified and re-introduced into the cell by artificial means.

A "structural gene" is that portion of a gene comprising a DNA segment encoding a protein, polypeptide or a portion thereof, and excluding the 5' sequence which drives the initiation of transcription. The structural gene may alternatively encode a nontranslatable product. The structural gene may be one which is normally found in the cell or one which is not normally found in the cell or cellular location wherein it is introduced, in which case it is termed a "heterologous gene". A heterologous gene may be derived in whole or in part from any source known to the art, including a bacterial genome or episome, eukaryotic, nuclear or plasmid DNA, cDNA, viral DNA or chemically synthesized DNA. A structural gene may contain one or more modifications which could effect biological activity or the characteristics, the biological activity or the chemical structure of the expression product, the rate of expression or the manner of expression control. Such modifications include, but are not limited to, mutations, insertions, deletions and

substitutions of one or more nucleotides. The structural gene may constitute an uninterrupted coding sequence or it may include one or more introns, bounded by the appropriate splice junctions. The structural gene may be translatable or non-translatable, including in an anti-sense orientation. The structural gene may be a composite of segments derived from a plurality of sources (naturally occurring or synthetic, where synthetic refers to DNA that is chemically synthesized).

"Divergent orientation" refers to an arrangement where sequences are pointing away from each other or in opposite directions in their direction of transcription.

"Derived from" is used to mean taken, obtained, received, traced, replicated or descended from a source (chemical and/or biological). A derivative may be produced by chemical or biological manipulation (including, but not limited to, substitution, addition, insertion, deletion, extraction, isolation, mutation and replication) of the original source.

"Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled in vitro. Manual chemical synthesis of DNA may be accomplished using well established procedures (Caruthers, Methodology of DNA and RNA Sequencing, (1983), Weissman (ed.), Praeger Publishers, New York, Chapter 1); automated chemical synthesis can be performed using one of a number of commercially available machines.

Two polynucleotides or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman

Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection.

The terms "substantial identity" or "substantial sequence identity" as applied to nucleic acid sequences and as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, and more preferably at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 25-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison. The reference sequence may be a subset of a larger sequence.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence-dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5°C to about 20°C, usually about 10°C to about 15°C, lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is about 0.02 molar at pH 7 and the temperature is at least about 60°C. For instance in

a standard Southern hybridization procedure, stringent conditions will include an initial wash in 6xSSC at 42 °C followed by one or more additional washes in 0.2xSSC at a temperature of at least about 55°C, typically about 60°C and often about 65°C.

Nucleotide sequences are also substantially identical for purposes of this invention when the polypeptides which they encode are substantially identical. Thus, where one nucleic acid sequence encodes essentially the same polypeptide as a second nucleic acid sequence, the two nucleic acid sequences are substantially identical, even if they would not hybridize under stringent conditions due to silent substitutions permitted by the genetic code (see, Darnell et al. (1990) Molecular Cell Biology, Second Edition Scientific American Books W. H. Freeman and Company New York for an explanation of codon degeneracy and the genetic code).

Protein purity or homogeneity can be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

As used herein, the term "cis" is used in reference to the presence of nucleic acid signal binding elements on a chromosome. The term "cis-acting" is used in reference to the controlling effect of a regulatory nucleic acid element on a gene. For example, enhancers and promoters may include cis acting control elements which may affect transcription.

As used herein, the term "vector" is used in reference to nucleic acid molecules that transfer DNA segment(s) into a cell. A vector may act to replicate DNA and may reproduce independently in a host cell. The term "vehicle" is sometimes used interchangeably with "vector."

The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, often along with other sequences. Eucaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals.

As used herein, the term "TATA element" or "TATA box" is used in reference to a segment of DNA, located approximately 19-27 base pairs upstream from the transcription start point of eucaryotic structural genes, to which RNA polymerase binds. The TATA box is approximately 7 base pairs in length, often comprising as one example, the sequence "TATAAAA" or "TATATAA". The TATA box is also sometimes referred to as the "Hogness box."

The term "CAAT box" or "CAAT element" refers to a conserved DNA sequence located upstream from the TATA box or the transcription start point of eucaryotic structural genes, to which RNA polymerase binds.

Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis, T. et al., Science 236:1237 (1987)). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells, plants and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a

broad host range while others are functional in a limited subset of cell types (for review see Voss, S. D. et al., Trends Biochem. Sci., 11:287 (1986) and Maniatis, T. et al., supra (1987)).

As used herein the term "transgene" refers to any gene that is not normally present in a particular host.

"Expressible coding sequence", as used herein, refers to a DNA sequence that serves as a template for the synthesis gene products or polypeptides. "Non-expressible coding sequence" refers to any DNA sequences that direct the synthesis of non-translatable transcripts, including antisense mRNA.

Core Promoters

In an important aspect, the BCPC of the present invention includes at least two core promoters. Structurally, the term "core promoter", as used herein, may correspond to, but not limited to, a DNA sequence of about 50 bp to about 100 bp in length. The DNA sequence may contain at least a TATA-box consensus element and the Initiator (INR), and preferably a TATA-box consensus element, the INR and at least one cis-acting element such as the CAAT-box or the *as-1* element (Benfey and Chua, Science 250:959-966 (1990)). A core promoter may be commonly isolated from DNA sequences immediately upstream of a transcription start site (TSS) or synthesized chemically according to pre-determined DNA sequence information.

Functionally, the term "core promoter", as used herein, is defined by its capability to direct the precise initiation and synthesis of transcripts from an operably linked nucleic acid sequence at a minimum activity level that can be detected by using currently available gene transcription analysis methods, including reverse transcriptase-polymerase chain reaction assay (RT-PCR), nucleic acid hybridization techniques, DNA-protein binding assays and in vitro and/or in vivo gene

expression analysis approaches using living cells (Wefald, et al., Nature 344:260-262 (1990); Benfey and Chua, Science 250:959-966 (1990); Patikoglou and Burley, annu. Rev. Biophys. Biomol. Struct. 26:289-325 (1997)). In one aspect, the core promoters of the invention have a sequence homology where promoter sequences have a homology when compared to each other of at least about 30% and include at least 5 bp identical contiguous nucleotides within the core promoter region.

Both structural and functional features of various core promoters have been previously studied extensively and described in great details in literature (Kollmar and Farnham, Proc. Exp. Biol. Med. 203:127-139 (1993); Orphanides, et al. Genes and Dev. 10:2657-2683 (1996); Roeder, Trends Biochem. Sci. 21:327-335 (1996); Tjian, Philos. Trans. R. Soc. Lond. B. Biol. Sci. 351:491-499 (1996)).

A core promoter is generally referred to as a DNA sequence that is directly located upstream of a nucleic acid sequence that is to be transcribed. However, in a BDPC said nucleic acid sequence may be either upstream or downstream from a core promoter. The nucleic acid sequence to be transcribed may be either translatable or non-translatable and may further include an open reading frame or coding sequence.

The TATA-box and the INR are the two key elements present in a core promoter, both of which play an important role in determining the TSS position and in initiating basal transcription. The consensus sequence for the TATA-box may comprise TATA(A/T)A(A/T) and the INR has the consensus YYAN(T/A)YY, where the underlined A indicates the TSS. According to observations from numerous cloned gene promoters, abundantly expressed genes generally contain a strong TATA-box in their core promoter, while most housekeeping genes, including oncogenes and those encoding growth factors and

transcription factors, may often contain no TATA-box in their core promoter. In some strong core promoters, other cis-acting elements, including the CAAT-box and the *as-1* element, are frequently found to be overlapped within the core promoter DNA sequence. For instance, the core promoter of the CaMV 35S promoter was defined experimentally to be a sequence ranging from +1 to -90. This fragement contains the TATA-box consensus (TATATAA), two CAAT-box elements and two *as-1* elements (Fang, et al. Plant Cell 1:141-150 (1989); Benfey, et al. EMBO J.9:1677-1684 (1990); Benfey and Chua, Science 250:959-966 (1990)).

Core promoters have a unique structure and organization at the DNA level. Core promoters in a BDPC may have substantial sequence identity or in one aspect of the invention, be identical. In another aspect, the core promoters of the invention have a sequence homology where promoter sequences have a homology of at least about 30% and include in separate aspects of the invention, at least 5, 10 or 20 bp identical contiguous nucleotides within the core promoter region. In another aspect, the core promoters have a sequence homology where promoter sequences have a homology of at least about 40% and include in separate aspects of the invention, at least 5, 10 or 20 identical contiguous nucleotides within the core promoter region. In another aspect, the core promoters have a sequence homology where promoter sequences have a homology of at least about 50% and include in separate aspects of the invention, at least 5, 10 or 20 identical contiguous nucleotides within the core promoter region.

Studies of protein-DNA interactions indicated that the DNA sequence for a core promoter provides critical binding elements and anchoring points essential for the formation of a productive transcription initiation subcomplex that comprises the RNA polymerase II (RNAPII),

numerous transcription factors (TFIIA, TFIIB, TFIID, CIFs, TAFs) and the TATA-binding protein (TBP) (see review by Zhang, Genome Res. 8:319-326 (1998)). Accordingly, it is easily recognized that a core promoter is one of the prerequisite components in the transcriptional machinery and plays an important role in supporting the precise initiation and synthesis of transcripts.

Sources of core promoters include but are not limited to CaMV 35S, CsVMV, ACT2, PRB1B, octopine synthase promoter, nopaline synthase promoter, manopine synthetase promoter, beta-conglycinin promoter, phaseolin promoter, ADH promoter, heat-shock promoters, developmentally regulated promoters, and tissue specific promoters.

Modified Enhancer Complex

The present invention includes a modified enhancer region, to which two core promoters are fused upstream and downstream thereof to form a BDPC. In another aspect of the invention, the enhancer sequences may have substantial sequence identity or may in one aspect include at least two identical enhancer sequences that are arranged in a tandem orientation. Alternatively, the enhancers of the invention have a sequence homology where enhancer sequences have a homology of at least about 30% and include at least 5 bp identical contiguous nucleotides within the enhancer sequence. More specifically, the 3' end of the first enhancer sequence is linked to the 5' end of the second sequence to form a modified enhancer region in a BDPC.

In yet another aspect of the present invention, each repeated enhancer sequence in a modified enhancer region may correspond to a DNA sequence of about 100 bp to more than about 1.0 kbp in length. The choice for a particular repeat size is preferably based on the desired

transcriptional enhancement and the desired requirements for a specific transgene expression pattern controlled by a particular set of cis-acting elements contained within the enhancer DNA sequence.

In yet another aspect, within a modified enhancer region there may be any number of cis-acting elements that are fully functional to the core promoters used in a BDPC. The cis-acting elements are functional, meaning capable of modulating, including enhancing or down-regulating, the initiation and synthesis of transcripts from a transgene containing either expressible or non-expressible coding sequences.

A modified enhancer region in a BDPC as used herein, may comprise at least two, more than two, or multiple of two, such as four and six, repeated enhancer sequences. If four enhancer repeat sequences are to be used to form a four-unit modified enhancer region in a BDPC, two enhancer sequences are first placed in tandem to form one enhancer array. Two different enhancer arrays made from a total of four repeat sequences will be then fused together in an opposite or back-to-back orientation. More specifically, transcription in the upstream direction may occur on the bottom strand whereas transcription in the downstream direction may occur on the top strand. Likewise, in the case where six enhancer sequences are to be chosen to construct a six-unit modified enhancer region in BDPC, three sequences are first arranged to form an array of tandem repeats. The two different enhancer arrays are finally fused together in a back-to-back orientation to form a six-unit modified enhancer region for use in a BDPC.

The sequence length of all repeated enhancer sequences within one enhancer array may be asymmetrical. As used herein, asymmetrical means that enhancer sequences are at least 10 bp either longer or shorter than the unit length of the enhancer units within the

other enhancer array, as used in either a four- or six-unit modified enhancer region. The use of asymmetric enhancer arrays in a four- or six-unit modified enhancer region is preferred to prevent the formation of a perfect palindromic sequence containing overly long (>100 bp) repeated sequences, which may affect stability during DNA manipulation and cloning processes (Allers and Leach, J. Mol. Biol. 252:72-85 (1995); Nasar et al., Mol. Cell. Biol. 20:3449-3458 (2000)).

The term "enhancer" has been previously defined (Khoury and Gruss, Cell 33:313-314 (1983) and extensively used to describe any DNA sequence with a size ranging from approximately 100 bp to over 2.0 kbp. According to studies of eukaryotic promoters, enhancers are commonly isolated from sequences located upstream or downstream of a core promoter and contain numerous cis-acting elements important for transcription regulation. In an important aspect, enhancers function to modulate, including either enhance or limit, the transcriptional activity of the core promoter in an orientation- and/or position-independent fashion. Transcriptional control or regulation of temporal- and spatial-specific gene expression in all eukaryotes is primarily associated with the presence of functional cis-acting elements within enhancers and is the results of interplay between these regulatory elements and cellular factors in host cells.

Over the years, numerous enhancers have been isolated form organisms ranging from viruses to higher mammals. For instance, in higher plants enhancers regulating gene expression in vegetative tissues, xylem and vascular tissues, roots, flowers, fruits and seeds, as well as gene expression in response to biotic and abiotic stresses, have been isolated and well characterized (see reviews by Edwards and Coruzzi, Annu Rev. Genet. 24:275-303 (1990); Guilfoyle, Genetic Engineering Vol. 19, pps. 15-47 (1997)). Many of these

isolated enhancers have been utilized in efforts to provide regulated control of transgene expression in host and non-host organisms.

Accordingly, in an important aspect of the present invention, all enhancers isolated thus far can be utilized to construct a modified enhancer region for use in a BDPC to effect transgene expression based on the regulatory information contained in the enhancer of choice. Functional enhancers that are chemically synthesized based on predetermined sequence information may also be used in the construction of a modified enhancer region as described in the present invention. The use of repeated enhancers in a modified enhancer region does not alter the gene expression pattern, but primarily provides a unique means to achieve transcriptional enhancement.

DNA can undergo dynamic conformational changes under many circumstances. Certain types of DNA sequences, including tandem repeats, reversed repeats, repetitive sequence arrays, and symmetrical or asymmetrical palindromic sequences, are conducive to the formation of so-called alternative DNA conformations, such as DNA bending, cruciform structures, DNA loops, DNA haripins, DNA 4-way junction structures, DNA triplexes and so forth (Perez et al., Ann. Rev. Microbiol. 51:593-628 (1997); Selker, Cell 97:157-160 (1999); Gaillard et al., BMC Biochem and Struct. Biol. 1:1 (2000); Caddle et al., J. Mol. Biol. 211:19-33 (1990); Courey et al. J. Mol. Biol. 202:35-43 (1988); Spink et al. PNAS 92:10767-10771 (1995); Moore et al. PNAS 96:1504-1509 (1999); Collin et al. NAR 28:3381-3391 (2000)). In some cases, alternative DNA conformations can be derived from intrinsic bonding interactions between nucleic acid residues contained in a unique DNA sequence; in other cases, they may be induced and/or augmented by the interplay between DNA sequence elements and DNA-binding factors (Pil et al. PNAS

90:9465-9 (1993); Wolfe et al. Chem Biol. 2:213-221 (1995); Slama-Schwok et al. NAR 25:2574-81 (1997)). Alternative DNA conformations within eukaryotic enhancers and promoters have been demonstrated to provide important architectural elements, complex signal interaction devices and efficacious molecular environments for DNA-protein interactions that may result in the formation of productive transcriptional machinery (Perz et al. Ann. Rev. Microbiol. 51:593-628 (1997)).

In one aspect, the present invention is intended to introduce into a BDPC an enhancer region modified to contain two tandem repeat(s) of substantially identical enhancer sequences and two core promoters with a high degree of sequence homology placed in opposite orientation on either side of the modified enhancer region. Although any particular helical structure or alternative conformation associated with a BDPC of the present invention needs to be determined by using molecular techniques available in the art, the significant enhancement of transcriptional activity observed from the use of a BDPC suggests the involvement of unique DNA structural geometry that provides a favorable molecular environment for productive interactions between DNA sequence elements within enhancer and core promoters and transcriptional factors present in host cells. Such interactions eventually lead to the onset of synergistically improved transcription from both core promoters.

Transgene Silencing

In another important aspect, the BDPC of the present invention is effective for decreasing the occurrence of gene silencing resulting from loss of promoter function due to methylation and the like. Changes in DNA structure can trigger the onset of gene silencing. Multiple copies of a gene and inverted gene repeats are

vulnerable to DNA methylation modifications that lead to transcriptional silencing (Selker, Cell 97:157-160 (1999)). Tandem repeats of integrated genes can be recognized and modified at the DNA level by host factors (Finnegan et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:223-247 (1998): Kumpatla et al., TIBS 3:97-104 (1998)). A cruciform structure derived from DNA repeats is effectively modified by a mammalian methyltransferase (Smith et al., J. Mol. Biol. 243:143-151 (1994)). However, many cases of transgene silencing derived from repeated sequences involves coding regions (Selker, Cell 97:157-160 (1999); Finnegan et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:223-247 (1998)). BDPCs of the present invention support stable and high levels of transgene expression even though repeated DNA sequences were present within the BDPC region.

Use of BDPCs

In another aspect of the invention, vectors that include a BDPC as described in this invention can be used to express foreign genes in mammalian cells and especially in plant cells that include dicots and monocots. More specifically, dicots include but are not limited to tobacco, grapes, soybeans, legumes, rapeseed, cotton, sunflower, tomatoes, potatoes, sugar beets, alfalfa, cloves and peanuts. Monocots include but are not limited to maize, wheat, sorghum, oats, rye, barley, rice, millets, sugar cane and grasses.

Several techniques exist for introducing foreign genetic material into plant cells, and for obtaining plants that stably maintain and express the introduced gene. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (US Patents 4,945,050 to Cornell and 5,141,131 to DowElanco). Plants may be transformed using Agrobacterium technology, see US Patent 5,177,010 to

University of Toledo, 5,104,310 to Texas A&M, European Patent Application 0131624B1, European Patent Applications 120516, 159418B1, European Patent Applications 120516, 159418B1 and 176,112 to Schilperoot, US Patents 5,149,645, 5,469,976, 5,464,763 and 4,940,838 and 4,693,976 to Schilperoot, European Patent Applications 116718, 290799, 320500 all to MaxPlanck, European Patent Applications 604662 and 627752 to Japan Tobacco, European Patent Applications 0267159, and 0292435 and US Patent 5,231,019 all to Ciba Geigy, US Patents 5,463,174 and 4,762,785 both to Calgene, and US Patents 5,004,863 and 5,159,135 both to Agracetus. Other transformation technology includes whiskers technology, see U.S. Patents 5,302,523 and 5,464,765 both to Zeneca. Electroporation technology has also been used to transform plants, see WO 87/06614 to Boyce Thompson Institute, 5,472,869 and 5,384,253 both to Dekalb, WO9209696 and WO9321335 both to PGS. All of these transformation patents and publications are incorporated by reference. In addition to numerous technologies for transforming plants, the type of tissue which is contacted with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue type I and II, hypocotyl, meristem, and the like. Almost all plant tissues may be transformed during dedifferentiation using appropriate techniques within the skill of an artisan.

Foreign genetic material introduced into a plant may include a selectable marker. The preference for a particular marker is at the discretion of the artisan, but any of the following selectable markers may be used along with any other gene not listed herein which could function as a selectable marker. Such selectable markers include but are not limited to aminoglycoside phosphotransferase gene of transposon Tn5 (Aph II) which encodes resistance to the antibiotics kanamycin, neomycin

and G418, as well as those genes which code for resistance or tolerance to glyphosate; hygromycin; methotrexate; phosphinothricin (bar); imidazolinones, sulfonylureas and triazolopyrimidine herbicides, such as chlorosulfuron; bromoxynil, dalapon and the like.

In addition to a selectable marker, it may be desirous to use a reporter gene. In some instances a reporter gene may be used without a selectable marker. Reporter genes are genes which are typically not present or expressed in the recipient organism or tissue. The reporter gene typically encodes for a protein which provide for some phenotypic change or enzymatic property. Examples of such genes are provided in K. Weising et al. Ann. Rev. Genetics, 22, 421 (1988), which is incorporated herein by reference. Preferred reporter genes include without limitation glucuronidase (GUS) gene and GFP genes.

Once introduced into the plant tissue, the expression of the structural gene may be assayed by any means known to the art, and expression may be measured as mRNA transcribed, protein synthesized, or the amount of gene silencing that occurs (see U.S. Patent No. 5,583,021 which is hereby incorporated by reference). Techniques are known for the in vitro culture of plant tissue, and in a number of cases, for regeneration into whole plants (EP Appln No. 88810309.0). Procedures for transferring the introduced expression complex to commercially useful cultivars are known to those skilled in the art.

Once plant cells expressing the gene under control of a bidirectional promoter are obtained, plant tissues and whole plants can be regenerated therefrom using methods and techniques well-known in the art. The regenerated plants are then reproduced by conventional means and the introduced genes can be transferred to

other strains and cultivars by conventional plant breeding techniques.

The following examples illustrate methods for carrying out the invention and should be understood to be illustrative of, but not limiting upon, the scope of the invention which is defined in the appended claims.

EXAMPLES

EXAMPLE 1: Preparation of Transformation Vectors

Two transformation vectors were constructed as illustrated in Fig. 13. Firstly, a green fluorescent protein (GFP) expression cassette was constructed. This cassette was composed of an EGFP (Clontech Laboratories, Inc., Palo Alto, CA) under the control of a core promoter (-90 to +1) (Benfey et al., Science 250:959-966 (1989)), and the terminator and polyadenylation signal of CaMV 35S transcript. This expression cassette was then isolated as a *HindIII* fragment and inserted into the *5' HindIII* site of the T-DNA region of a binary vector pBI434 (Li et al., Transgenic Crop I. Biotechnology in Agriculture and Forestry, vol. 46 (1999)). This binary vector contained a GUS-NPTII fusion gene (Dalta et al., Gene 101:239-246 (1991)) under the control of an enhanced double CaMV 35S promoter (Kay et al., Science 236:1299-1302 (1987)) followed by a 5' nontranslated leader sequence of alfalfa mosaic virus (AMV) and with a terminator and polyadenylation signal of the nopaline synthase gene of *Agrobacterium*. Two transformation vectors were obtained depending on the orientation of insertion. In vector p201, the GFP expression cassette was in a tandem orientation relative to the GUS-NPTII expression unit. Secondly, the GFP expression cassette in vector p201R was in a divergent orientation leading to the formation of a BDPC in this vector. In the BDPC, two identical core promoters of the CaMV 35S transcript were located on

either side of a duplicated enhancer region [2X (-363 to -91)] resulting in a total size of 736 bp in length (Fig. 2).

EXAMPLE 2: Transformation of Somatic Embryos of Grape

Binary vectors p201 and p201R were both introduced into *A. tumefaciens* strain EHA105 and subsequently used to transform somatic embryos (SE) of grape (*Vitis vinifera* cv. Thompson Seedless). Expression of the EGFP gene was monitored after transformation using a stereomicroscope equipped with a fluorescence illuminator and GFP filter system. GUS expression was quantitatively determined by using a fluorogenic assay as described by Jefferson (Plant Mol. Biol. Rep. 5:387-405).

As shown in Fig. 14, the differential effects of vectors p201 and p201R on the level of GFP expression were readily noticeable one week after transformation. SE transformed with p201 fluoresced only slightly, while SE transformed with p201R fluoresced brightly. Microscopic observation of the SE revealed that the density of GFP-expressing cells on the surface of transformed SE was similar for both vector treatments. These results indicated that the observed difference in the level of GFP expression between these two vectors was the result of the difference in strength of the promoters used to control EGFP gene expression (Fig. 13). The reduced level of GFP expression in SE following transformation with p201, as opposed to p201R, suggests that the transcriptional activity of the same core promoter can be dramatically increased by using a BDPC.

In addition to enhancing gene expression, use of BDPC increased transformation efficiency based on assays of transient GFP expression (Fig. 15). In two independent experiments, transformation using p201R resulted in an increase of about 19% and about 44%,

respectively, in the number of GFP-expressing SE, when compared to p201.

To examine the effect of the BDPC on the downstream core promoter, GFP-expressing SE were selected and further analyzed for GUS expression using a fluorogenic assay. The results illustrated in Fig. 16 indicate that GUS activity in SE transformed using p201R was consistently about 40% higher than the GUS activity detected in SE transformed using p201.

Transgenic embryos and plants were subsequently recovered from the SE transformed using p201R. A consistently high level of GFP expression was observed throughout their subsequent developmental stages and in various plant tissues (Fig. 17), with a similar gene expression pattern achieved by using the CaMV 35S promoter as reported previously (Benfey et al., Science 250:959-966 (1989)). This suggests that the induced enhanced gene expression is spatially and temporally stable in transgenic grape plants.

Experimental data obtained indicate that the BDPC present in p201R is capable of significantly elevating the level of expression of both transgenes (EGFP and GUS), as compared to that obtained using p201, which contains a conventional promoter/transgene configuration. This gene expression enhancement is possibly attributable to an improvement in the structural configuration of the BDPC that results in increased promoter activity.

The addition of a second core promoter to the upstream region of the double promoter in a tandem orientation relative to the downstream core promoter, in p201 constituted an array of tandem repeats of promoter sequences within the T-DNA which induces gene silencing (Kumpatla et al., TIBS 3:97-104 (1998)).

EXAMPLE 3: Quantification of Transgene Expression

To determine quantitatively the transgene expression under control of the upstream core promoter in a BDPC as described in the invention, transformation vectors pLC501T and pLC501R were constructed. As illustrated in Fig. 24, the T-DNA regions of both pLC501T and pLC501R were essentially identical to that of pLC201 and pLC201R, respectively, as shown in Fig. 13, except that the positions of the GUS gene and the EGFP/NPTII gene were switched around, and both transgenes were fused to the terminator of CaMV 35S transcript.

Both pLC501T and pLC501R were introduced into *A. tumefaciens* and subsequently used in transformation of grape SE (cv. Thompson Seedless) as described in Example 2. In this experiment, transformation vector pBI434 containing no BDPC but a GUS/NPTII fusion gene under control of an enhanced double CaMV 35S promoter was also included for GUS activity comparison. Fig. 25 shows GUS activity in SE transformed with various vectors. Noticeably, the core promoter in pLC501T only supported a minimum level of GUS expression (8 pmol MU/mg for 60 min), while a huge increase in GUS expression was observed from SE transformed with pLC501R (1774 pmol MU/mg for 60 min). In other words, up to 220-fold increase in GUS activity was achieved by using pLC501R in which the GUS gene was under the control of the upstream core promoter in a BDPC setting, as compared to the GUS activity derived from the same core promoter without a BDPC configuration (pLC501T). In addition, the GUS activity derived from the upstream core promoter of the BDPC in pLC501R increased by 2-fold, as compared to GUS activity resulted from pBI434, which only contained an enhanced double CaMV 35S promoter. These data, together with observations described in Example 2, clearly demonstrate that a BDPC as described in the invention is effective for achieving stable and significantly high

levels of transgene expression enhancement from both core promoters.

EXAMPLE 4: Quantification of Transgene Expression under 4-Enhancer-Containing BDPC

To investigate transgene expression directed by a BDPC containing 4 enhancers, two transformation vectors pLC903T and pLC903R were constructed. As shown in Fig. 26, both vectors contained an EGFP expression unit and a GUS-containing expression unit. The two expression units were under the control of a similar enhanced double CaMV 35S promoter with a slightly different sequence length of enhancers. In pLC903T the two expression units were placed in a tandem orientation. The two expression units in pLC903R were placed in a divergent (back-to-back) orientation, thus resulting in the formation of a 4-enhancer-containing BDPC for the expression of both EGFP and GUS genes. The BDPC configuration in pLC903R is basically similar to that as illustrated in Fig. 3.

Both pLC903T and pLC903R were introduced into *A. tumefaciens* and subsequently used in transformation of grape SE along with a control transformation vector pBI434 as previously described in Examples 2 and 3. The level of GUS expression in transformed SE was determined subsequently and the averaged results from three independent experiments were summarized in Fig. 27. In these experiments, GUS activity obtained from 30-min reactions was used for data conversion. Results indicated that there was no GUS-specific activity in non-transformed SE (CK-0.3 pmol MU/mg/min). Surprisingly , the GUS activity obtained from SE transformed with pLC903T was about half of that observed from pLC434 (36 vs. 65.4 pmol MU/mg/min), even though the GUS expression unit in both vectors was identical and was controlled by the same enhanced double CaMV 35S promoter. The reduction in GUS expression observed from the use of

pLC903T could be accounted for by the possible interference of terminator sequences (35S-31) in the upstream region of the GUS expression unit in pLC903T. On the contrary, an increase in GUS activity by almost 10-fold was observed in SE transformed with pLC903R, which contains a 4-enhancer-containing BDPC in the upstream region of the core promoter, as compared to the GUS activity from pBI434, which only contained an enhanced double CaMV35S promoter (638.2 vs. 65.4 pmol MU/mg/min). The dramatic increase in GUS expression by using transformation vector pLC903R further demonstrated the significant enhancement of trangene expression from the use of unique BDPC promoter configuration as elucidated in this invention.

Numerous modifications and variations in practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing detailed description of the invention. Consequently, such modifications and variations are intended to be included within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A bidirectional promoter complex comprising:

a modified enhancer region that includes at least two enhancer sequences; and

at least two core promoters,

the core promoters being on either side of the modified enhancer region in a divergent orientation.

2. The bidirectional promoter complex of claim 1 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

3. The bidirectional promoter complex of claim 1 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

4. The bidirectional promoter complex of claim 1 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

5. The bidirectional promoter complex of claim 1 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

6. The bidirectional promoter complex of claim 1 wherein the core promoters are fused to either end of the modified enhancer region in a divergent orientation.

7. The bidirectional promoter complex of claim 1 wherein each core promoter includes a TATA-box concensus element and an Initiator.

8. The bidirectional promoter complex of claim 7 wherein each core promoter further includes at least one cis-acting element.

9. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

10. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

11. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

12. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

13. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

14. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

15. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

16. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

17. The bidirectional promoter complex of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

18. A vector comprising a bidirectional promoter complex, the bidirectional promoter complex including a modified enhancer region and at least two core promoters,

the core promoters being on either side of the modified enhancer complex in a divergent orientation.

19. The vector of claim 18 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

20. The vector of claim 18 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

21. The vector of claim 18 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

22. The vector of claim 18 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

23. The vector of claim 18 wherein the core promoters are fused to either end of the modified enhancer region in a divergent orientation.

24. The vector of claim 18 wherein each core promoter includes a TATA-box concensus element and an Initiator.

25. The vector of claim 18 wherein each core promoter further includes at least one cis-acting element.

26. The vector of claim 18 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

27. The vector of claim 18 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

28. The vector of claim 18 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

29. The vector of claim 18 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

30. The vector of claim 18 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

31. The vector of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

32. The vector of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

33. The vector of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

34. The vector of claim 1 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

35. A eukaryotic cell transfected with a vector, the vector comprising a bidirectional promoter complex, the bidirectional promoter complex including a modified enhancer region and at least two core promoters, the core promoters being on either side of the modified enhancer region in a divergent orientation.

36. The eukaryotic cell of claim 35 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

37. The eukaryotic cell of claim 35 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

38. The eukaryotic cell of claim 35 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

39. The eukaryotic cell of claim 35 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

40. The eukaryotic cell of claim 35 wherein the core promoters are fused to either end of the modified enhancer region in a divergent orientation.

41. The eukaryotic cell of claim 35 wherein each core promoter includes a TATA-box concensus element and an Initiator.

42. The eukaryotic cell of claim 41 wherein each core promoter further includes at least one cis-acting element.

43. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

44. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

45. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

46. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

47. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

48. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

49. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

50. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

51. The eukaryotic cell of claim 35 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

52. A transgenic plant comprising plant cells that have been transformed with a vector that includes a bidirectional promoter complex, the bidirectional promoter complex including a modified enhancer region and at least two core promoters, the core promoters being on either side of the modified enhancer region in a divergent orientation.

53. The transgenic plant of claim 52 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

54. The transgenic plant of claim 52 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

55. The transgenic plant of claim 52 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

56. The transgenic plant of claim 52 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

57. The transgenic plant of claim 52 wherein the core promoters are fused to either end of the modified enhancer region in a divergent orientation.

58. The transgenic plant of claim 52 wherein each core promoter includes a TATA-box concensus element and an Initiator.

59. The transgenic plant of claim 58 wherein each core promoter further includes at least one cis-acting element.

60. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

61. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

62. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

63. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

64. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

65. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

66. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

67. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

68. The transgenic plant of claim 58 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

69. A plant seed having in its genome an inheritable genetic complex, the inheritable genetic complex comprising a bidirectional promoter complex, the bidirectional promoter complex including a modified enhancer enhancer regions and at least two core promoters, the core promoters being on either side of the modified enhancer region in a divergent orientation.

70. The plant seed of claim 69 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

71. The plant seed of claim 69 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

72. The plant seed of claim 69 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

73. The plant seed of claim 69 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

74. The plant seed of claim 69 wherein the core promoters are fused to either end of the modified enhancer region in a divergent orientation.

75. The plant seed of claim 69 wherein each core promoter includes a TATA-box concensus element and an Initiator.

76. The plant seed of claim 75 wherein each core promoter further includes at least one cis-acting element.

77. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

78. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

79. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

80. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

81. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

82. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

83. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

84. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

85. The plant seed of claim 69 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

86. A method for improving transcription efficiency of transgenes, the method comprising inserting the transgene into a vector, the vector comprising a bidirectional promoter complex, the bidirectional promoter complex including a modified enhancer region and at least two core promoters, the core promoters being on either side of the modified enhancer region in a divergent orientation, the bidirectional promoter complex being effective for improving transcriptional efficiency of the transgene.

87. The method of claim 86 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

88. The method of claim 86 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

89. The method of claim 86 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

90. The method of claim 86 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

91. The method of claim 86 wherein each core promoter includes a TATA-box concensus element and an Initiator.

92. The method of claim 92 wherein each core promoter further includes at least one cis-acting element.

93. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

94. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

95. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

96. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

97. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

98. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

99. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

100. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

101. The method of claim 86 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

102. A method for producing one or more polypeptides, the method comprising inserting a transgene into a vector, the vector comprising a bidirectional promoter complex, the bidirectional promoter complex including a modified enhancer region and at least two core promoters, the core promoters being on either side of the modified enhancer complex in a divergent orientation, the bidirectional promoter complex being effective for improving transcriptional efficiency of the transgene.

103. The method of claim 102 wherein the modified enhancer region includes at least two tandem oriented enhancer sequences having substantial sequence identity.

104. The method of claim 102 wherein the modified enhancer region is constructed such that a 3' end of a first enhancer sequence is linked to a 5' end of a second enhancer sequence.

105. The method of claim 102 wherein the modified enhancer region includes a number of enhancer sequences which is a multiple of two.

106. The method of claim 102 wherein the core promoters have a sequence homology of about 30% and include at least about 5 base pairs of identical contiguous nucleotides.

107. The method of claim 102 wherein each core promoter includes a TATA-box concensus element and an Initiator.

108. The method of claim 107 wherein each core promoter further includes at least one cis-acting element.

109. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 1 and 2.

110. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 3 and 4.

111. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 5 and 6.

112. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 7 and 8.

113. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 9 and 10.

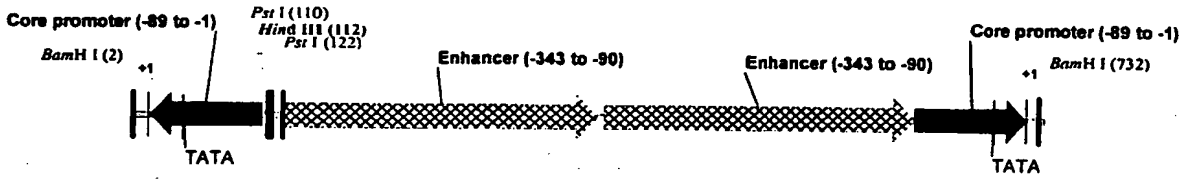
114. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 11 and 12.

115. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 13 and 14.

116. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 15 and 16.

117. The method of claim 102 wherein the bidirectional promoter complex includes SEQ. ID. Nos. 17 and 18.

Fig. 1

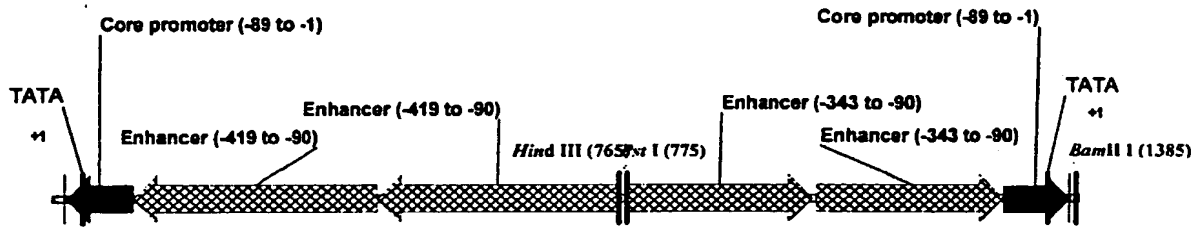


BDPC with 2 enhancers based on CaMV 35S promoter

736 bp

```
      BamHI
      ~~~~~~
  1   GGATCCAGCG TGTCCTCTCC AAATGAAATG AACTTCCTTA TATAGAGGAA GGGTCTTGCG AAGGATAGTG GGATTGTGCG
      CCTAGGTCGC ACAGGAGAGG TTTACTTTAC TTGAAGGAAT ATATCTCCTT CCCAGAACGC TTCCTATCAC CCTAACACGC
-----------------------------------------------------------------------------------------------
                                  PstI  HindIIIPstI
                                  ~~~~~~~~~~~~~~~~~~
 81   TCATCCCTTA CGTCAGTGGA GATACTGCAG AAGCTTCTGC AGTGAGACTT TTCAACAAAG GGTAATATCG GGAAACCTCC
      AGTAGGGAAT GCAGTCACCT CTATGACGTC TTCGAAGACG TCACTCTGAA AAGTTGTTTC CCATTATAGC CCTTTGGAGG
-----------------------------------------------------------------------------------------------
161   TCGGATTCCA TTGCCCAGCT ATCTGTCACT TCATCAAAAG GACAGTAGAA AAGGAAGGTG GCACCTACAA ATGCCATCAT
      AGCCTAAGGT AACGGGTCGA TAGACAGTGA AGTAGTTTTC CTGTCATCTT TTCCTTCCAC CGTGGATGTT TACGGTAGTA
-----------------------------------------------------------------------------------------------
241   TGCGATAAAG GAAAGGCTAT CGTTCAAGAT GCCTCTGCCG ACAGTGGTCC CAAAGATGGA CCCCCACCCA CGAGGAGCAT
      ACGCTATTTC CTTTCCGATA GCAAGTTCTA CGGAGACGGC TGTCACCAGG GTTTCTACCT GGGGGTGGGT GCTCCTCGTA
-----------------------------------------------------------------------------------------------
321   CGTGGAAAAA GAAGACGTTC CAACCACGTC TTCAAAGCAA GTGGATTGAT GTGATTGCAG TGAGACTTTT CAACAAAGGG
      GCACCTTTTT CTTCTGCAAG GTTGGTGCAG AAGTTTCGTT CACCTAACTA CACTAACGTC ACTCTGAAAA GTTGTTTCCC
-----------------------------------------------------------------------------------------------
401   TAATATCGGG AAACCTCCTC GGATTCCATT GCCCAGCTAT CTGTCACTTC ATCAAAAGGA CAGTAGAAAA GGAAGGTGGC
      ATTATAGCCC TTTGGAGGAG CCTAAGGTAA CGGGTCGATA GACAGTGAAG TAGTTTTCCT GTCATCTTTT CCTTCCACCG
-----------------------------------------------------------------------------------------------
481   ACCTACAAAT GCCATCATTG CGATAAAGGA AAGGCTATCG TTCAAGATGC CTCTGCCGAC AGTGGTCCCA AAGATGGACC
      TGGATGTTTA CGGTAGTAAC GCTATTTCCT TTCCGATAGC AAGTTCTACG GAGACGGCTG TCACCAGGGT TTCTACCTGG
-----------------------------------------------------------------------------------------------
561   CCCACCCACG AGGAGCATCG TGGAAAAAGA AGACGTTCCA ACCACGTCTT CAAAGCAAGT GGATTGATGT GATATCTCCA
      GGGTGGGTGC TCCTCGTAGC ACCTTTTTCT TCTGCAAGGT TGGTGCAGAA GTTTCGTTCA CCTAACTACA CTATAGAGGT
-----------------------------------------------------------------------------------------------
641   CTGACGTAAG GGATGACGCA CAATCCCACT ATCCTTCGCA AGACCCTTCC TCTATATAAG GAAGTTCATT TCATTTGGAG
      GACTGCATTC CCTACTGCGT GTTAGGGTGA TAGGAAGCGT TCTGGGAAGG AGATATATTC CTTCAAGTAA AGTAAACCTC
-----------------------------------------------------------------------------------------------
                BamHI
                ~~~~~~
721   AGGACACGCT GGATCC  Seq. ID No. 1
      TCCTGTGCGA CCTAGG  Seq. ID No. 2
-----------------------------------------------------------------------------------------------
```

Fig. 3



BDPC with 4 enhancers based on CaMV 35S promoter

1389 bp

FIG. 4

SnaBI

Seq. ID No. 3 1 TACGTACAGC GTGTCCTCTC CAAATGAAAT GAACTTCCTT ATATAGAGGA AGGGTCTTGC GAAGGATAGT GGGATTGTGC
Seq. ID No. 4 ATGCATGTCG CACAGGAGAG GTTTACTTTA CTTGAAGGAA TATATCTCCT TCCCAGAACG CTTCCTATCA CCCTAACACG

81 GTCATCCCTT ACGTCAGTGG AGATATCACA TCAATCCACT TGCTTTGAAG ACGTGGTTGG AACGTCTTCT TTTTCCACGA
CAGTAGGGAA TGCAGTCACC TCTATAGTGT AGTTAGGTGA ACGAAACTTC TGCACCAACC TTGCAGAAGA AAAAGGTGCT

161 TGCTCCTCGT GGGTGGGGGT CCATCTTTGG GACCACTGTC GGCAGAGGCA TCTTCAACGA TGGCCTTTCC TTTATCGCAA
ACGAGGAGCA CCCACCCCCA GGTAGAAACC CTGGTGACAG CCGTCTCCGT AGAAGTTGCT ACCGGAAAGG AAATAGCGTT

241 TGATGGCATT TGTAGGAGCC ACCTTCCTTT TCCACTATCT TCACAATAAA GTGACAGATA GCTGGGCAAT GGAATCCGAG
ACTACCGTAA ACATCCTCGG TGGAAGGAAA AGGTGATAGA AGTGTTATTT CACTGTCTAT CGACCCGTTA CCTTAGGCTC

321 GAGGTTTCCG GATATTACCC TTTGTTGAAA AGTCTCAATT GCCCTTTGGT CTTCTGAGAC TGTATCTTTG ATATTTTTGG
CTCCAAAGGC CTATAATGGG AAACAACTTT TCAGAGTTAA CGGGAAACCA GAAGACTCTG ACATAGAAAC TATAAAAACC

401 AGTAGACAAG TGTGTCGTGC TCCACCATGT TGATTCACAT CAATCCACTT GCTTTGAAGA CGTGGTTGGA ACGTCTTCTT
TCATCTGTTC ACACAGCACG AGGTGGTACA ACTAAGTGTA GTTAGGTGAA CGAAACTTCT GCACCAACCT TGCAGAAGAA

481 TTTCCACGAT GCTCCTCGTG GGTGGGGGTC CATCTTTGGG ACCACTGTCG GCAGAGGCAT CTTCAACGAT GGCCTTTCCT
AAAGGTGCTA CGAGGAGCAC CCACCCCCAG GTAGAAACCC TGGTGACAGC CGTCTCCGTA GAAGTTGCTA CCGGAAAGGA

561 TTATCGCAAT GATGGCATTT GTAGGAGCCA CCTTCCTTTT CCACTATCTT CACAATAAAG TGACAGATAG CTGGGCAATG
AATAGCGTTA CTACCGTAAA CATCCTCGGT GGAAGGAAAA GGTGATAGAA GTGTTATTTC ACTGTCTATC GACCCGTTAC

641 GAATCCGAGG AGGTTTCCGG ATATTACCCT TTGTTGAAAA GTCTCAATTG CCCTTTGGTC TTCTGAGACT GTATCTTTGA
CTTAGGCTCC TCCAAAGGCC TATAATGGGA AACAACTTTT CAGAGTTAAC GGGAAACCAG AAGACTCTGA CATAGAAACT

HindIIIPstI

721 TATTTTTGGA GTAGACAAGT GTGTCGTGCT CCACCATGTT GATAAGCTTC TGCAGTGAGA CTTTTCAACA AAGGGTAATA
ATAAAAACCT CATCTGTTCA CACAGCACGA GGTGGTACAA CTATTCGAAG ACGTCACTCT GAAAAGTTGT TTCCCATTAT

801 TCGGGAAACC TCCTCGGATT CCATTGCCCA GCTATCTGTC ACTTCATCAA AAGGACAGTA GAAAAGGAAG GTGGCACCTA
AGCCCTTTGG AGGAGCCTAA GGTAACGGGT CGATAGACAG TGAAGTAGTT TTCCTGTCAT CTTTTCCTTC CACCGTGGAT

881 CAAATGCCAT CATTGCGATA AAGGAAAGGC TATCGTTCAA GATGCCTCTG CCGACAGTGG TCCCAAAGAT GGACCCCCAC
GTTTACGGTA GTAACGCTAT TTCCTTTCCG ATAGCAAGTT CTACGGAGAC GGCTGTCACC AGGGTTTCTA CCTGGGGGTG

961 CCACGAGGAG CATCGTGGAA AAAGAAGACG TTCCAACCAC GTCTTCAAAG CAAGTGGATT GATGTGATTG CAGTGAGACT
GGTGCTCCTC GTAGCACCTT TTTCTTCTGC AAGGTTGGTG CAGAAGTTTC GTTCACCTAA CTACACTAAC GTCACTCTGA

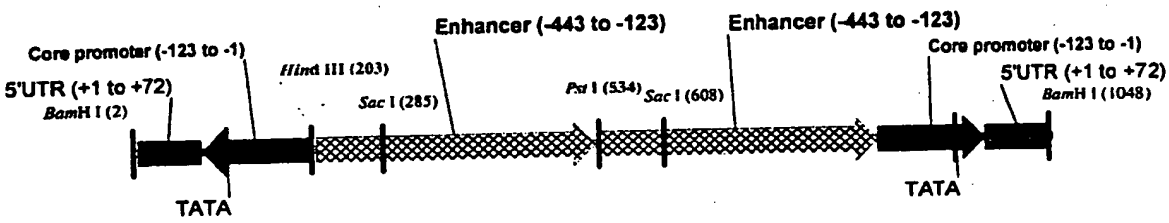
1041 TTTCAACAAA GGGTAATATC GGGAAACCTC CTCGGATTCC ATTGCCCAGC TATCTGTCAC TTCATCAAAA GGACAGTAGA
AAAGTTGTTT CCCATTATAG CCCTTTGGAG GAGCCTAAGG TAACGGGTCG ATAGACAGTG AAGTAGTTTT CCTGTCATCT

1121 AAAGGAAGGT GGCACCTACA AATGCCATCA TTGCGATAAA GGAAAGGCTA TCGTTCAAGA TGCCTCTGCC GACAGTGGTC
TTTCCTTCCA CCGTGGATGT TTACGGTAGT AACGCTATTT CCTTTCCGAT AGCAAGTTCT ACGGAGACGG CTGTCACCAG

1201 CCAAAGATGG ACCCCCACCC ACGAGGAGCA TCGTGGAAAA AGAAGACGTT CCAACCACGT CTTCAAAGCA AGTGGATTGA
GGTTTCTACC TGGGGGTGGG TGCTCCTCGT AGCACCTTTT TCTTCTGCAA GGTTGGTGCA GAAGTTTCGT TCACCTAACT

1281 TGTGATATCT CCACTGACGT AAGGGATGAC GCACAATCCC ACTATCCTTC GCAAGACCCT TCCTCTATAT AAGGAAGTTC
ACACTATAGA GGTGACTGCA TTCCCTACTG CGTGTTAGGG TGATAGGAAG CGTTCTGGGA AGGAGATATA TTCCTTCAAG

Fig. 5



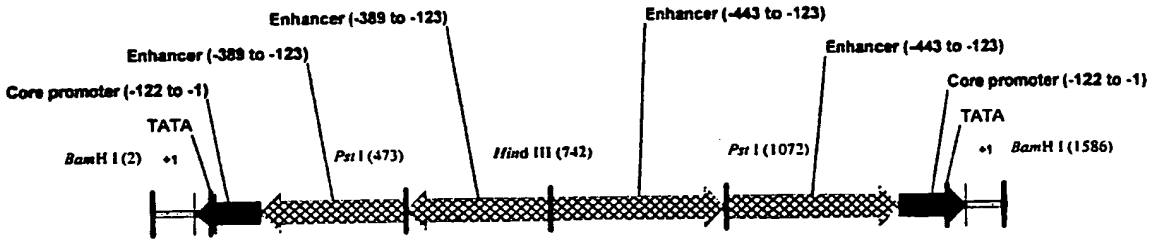
BDPC with 2 enhancers based on CsVMV promoter

1052 bp

Fig. 6

```
      BamHI
      ~~~~~~
  1   GGATCCACAA ACTTACAAAT TTCTCTGAAG TTGTATCCTC AGTACTTCAA AGAAAATAGC TTACACCAAA TTTTTTCTTG
      CCTAGGTGTT TGAATGTTTA AAGAGACTTC AACATAGGAG TCATGAAGTT TCTTTTATCG AATGTGGTTT AAAAAAGAAC
-----------------------------------------------------------------------------------------------
 81   TTTTCACAAA TGCCGAACTT GGTTCCTTAT ATAGGAAAAC TCAAGGGCAA AAATGACACG GAAAAATATA AAAGGATAAG
      AAAAGTGTTT ACGGCTTGAA CCAAGGAATA TATCCTTTTG AGTTCCCGTT TTTACTGTGC CTTTTTATAT TTTCCTATTC
-----------------------------------------------------------------------------------------------
                                                HindIII
                                                ~~~~~~
161   TAGTGGGGGA TAAGATTCCT TTGTGATAAG GTTACTTTCC GAAGCTTCCA GAAGGTAATT ATCCAAGATG TAGCATCAAG
      ATCACCCCCT ATTCTAAGGA AACACTATTC CAATGAAAGG CTTCGAAGGT CTTCCATTAA TAGGTTCTAC ATCGTAGTTC
-----------------------------------------------------------------------------------------------
                                              SacI
                                              ~~~~~~~
241   AATCCAATGT TTACGGGAAA AACTATGGAA GTATTATGTG AGCTCAGCAA GAAGCAGATC AATATGCGGC ACATATGCAA
      TTAGGTTACA AATGCCCTTT TTGATACCTT CATAATACAC TCGAGTCGTT CTTCGTCTAG TTATACGCCG TGTATACGTT
-----------------------------------------------------------------------------------------------
321   CCTATGTTCA AAAATGAAGA ATGTACAGAT ACAAGATCCT ATACTGCCAG AATACGAAGA AGAATACGTA GAAATTGAAA
      GGATACAAGT TTTTACTTCT TACATGTCTA TGTTCTAGGA TATGACGGTC TTATGCTTCT TCTTATGCAT CTTTAACTTT
-----------------------------------------------------------------------------------------------
401   AAGAAGAACC AGGCGAAGAA AAGAATCTTG AAGACGTAAG CACTGACGAC AACAATGAAA AGAAGAAGAT AAGGTCGGTG
      TTCTTCTTGG TCCGCTTCTT TTCTTAGAAC TTCTGCATTC GTGACTGCTG TTGTTACTTT TCTTCTTCTA TTCCAGCCAC
-----------------------------------------------------------------------------------------------
                                                    PstI
                                                    ~~~~~~~
481   ATTGTGAAAG AGACATAGAG GACACATGTA AGGTGGAAAA TGTAAGGGCT GCAGAAGGTA ATTATCCAAG ATGTAGCATC
      TAACACTTTC TCTGTATCTC CTGTGTACAT TCCACCTTTT ACATTCCCGA CGTCTTCCAT TAATAGGTTC TACATCGTAG
-----------------------------------------------------------------------------------------------
                                                SacI
                                                ~~~~~~
561   AAGAATCCAA TGTTTACGGG AAAAACTATG GAAGTATTAT GTGAGCTCAG CAAGAAGCAG ATCAATATGC GGCACATATG
      TTCTTAGGTT ACAAATGCCC TTTTTGATAC CTTCATAATA CACTCGAGTC GTTCTTCGTC TAGTTATACG CCGTGTATAC
-----------------------------------------------------------------------------------------------
641   CAACCTATGT TCAAAAATGA AGAATGTACA GATACAAGAT CCTATACTGC CAGAATACGA AGAAGAATAC GTAGAAATTG
      GTTGGATACA AGTTTTTACT TCTTACATGT CTATGTTCTA GGATATGACG GTCTTATGCT TCTTCTTATG CATCTTTAAC
-----------------------------------------------------------------------------------------------
721   AAAAAGAAGA ACCAGGCGAA GAAAAGAATC TTGAAGACGT AAGCACTGAC GACAACAATG AAAAGAAGAA GATAAGGTCG
      TTTTTCTTCT TGGTCCGCTT CTTTTCTTAG AACTTCTGCA TTCGTGACTG CTGTTGTTAC TTTTCTTCTT CTATTCCAGC
-----------------------------------------------------------------------------------------------
801   GTGATTGTGA AAGAGACATA GAGGACACAT GTAAGGTGGA AAATGTAAGG GCGGAAAGTA ACCTTATCAC AAAGGAATCT
      CACTAACACT TTCTCTGTAT CTCCTGTGTA CATTCCACCT TTTACATTCC CGCCTTTCAT TGGAATAGTG TTTCCTTAGA
-----------------------------------------------------------------------------------------------
881   TATCCCCCAC TACTTATCCT TTTATATTTT TCCGTGTCAT TTTTGCCCTT GAGTTTTCCT ATATAAGGAA CCAAGTTCGG
      ATAGGGGGTG ATGAATAGGA AAATATAAAA AGGCACAGTA AAAACGGGAA CTCAAAAGGA TATATTCCTT GGTTCAAGCC
-----------------------------------------------------------------------------------------------
961   CATTTGTGAA AACAAGAAAA AATTTGGTGT AAGCTATTTT CTTTGAAGTA CTGAGGATAC AACTTCAGAG AAATTTGTAA
      GTAAACACTT TTGTTCTTTT TTAAACCACA TTCGATAAAA GAAACTTCAT GACTCCTATG TTGAAGTCTC TTTAAACATT
-----------------------------------------------------------------------------------------------
          BamHI
          ~~~~~~~
1041  GTTTGTGGAT CC  Seq. ID No. 5
      CAAACACCTA GG  Seq. ID No. 6
-----------------------------------------------------------------------------------------------
```

Fig. 7



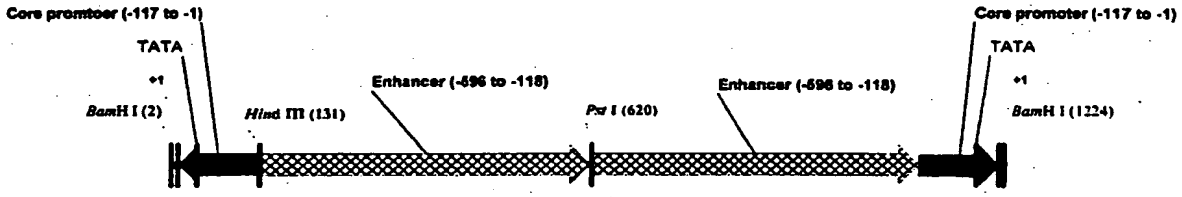
BDPC with 4 enhancers based on CsVMV promoter

1590 bp

```
      BamHI
      ~~~~~~
  1   GGATCCACAA ACTTACAAAT TTCTCTGAAG TTGTATCCTC AGTACTTCAA AGAAAATAGC TTACACCAAA TTTTTTCTTG
      CCTAGGTGTT TGAATGTTTA AAGAGACTTC AACATAGGAG TCATGAAGTT TCTTTTATCG AATGTGGTTT AAAAAAGAAC

 81   TTTTCACAAA TGCCGAACTT GGTTCCTTAT ATAGGAAAAC TCAAGGGCAA AAATGACACG GAAAAATATA AAAGGATAAG
      AAAAGTGTTT ACGGCTTGAA CCAAGGAATA TATCCTTTTG AGTTCCCGTT TTTACTGTGC CTTTTTATAT TTTCCTATTC

161   TAGTGGGGGA TAAGATTCCT TTGTGATAAG GTTACTTTCC GCCCTTACAT TTTCCACCTT ACATGTGTCC TCTATGTCTC
      ATCACCCCCT ATTCTAAGGA AACACTATTC CAATGAAAGG CGGGAATGTA AAAGGTGGAA TGTACACAGG AGATACAGAG

241   TTTCACAATC ACCGACCTTA TCTTCTTCTT TTCATTGTTG TCGTCAGTGC TTACGTCTTC AAGATTCTTT TCTTCGCCTG
      AAAGTGTTAG TGGCTGGAAT AGAAGAAGAA AAGTAACAAC AGCAGTCACG AATGCAGAAG TTCTAAGAAA AGAAGCGGAC

321   GTTCTTCTTT TTCAATTTCT ACGTATTCTT CTTCGTATTC TGGCAGTATA GGATCTTGTA TCTGTACATT CTTCATTTTT
      CAAGAAGAAA AAGTTAAAGA TGCATAAGAA GAAGCATAAG ACCGTCATAT CCTAGAACAT AGACATGTAA GAAGTAAAAA

                                                    SacI                    PstI
                                                    ~~~~~~                  ~~~~~~~
401   GAACATAGGT TGCATATGTG CCGCATATTG ATCTGCTTCT TGCTGAGCTC ACATAATACT TCCATAGCTG CAGCCCTTAC
      CTTGTATCCA ACGTATACAC GGCGTATAAC TAGACGAAGA ACGACTCGAG TGTATTATGA AGGTATCGAC GTCGGGAATG

481   ATTTTCCACC TTACATGTGT CCTCTATGTC TCTTTCACAA TCACCGACCT TATCTTCTTC TTTTCATTGT TGTCGTCAGT
      TAAAAGGTGG AATGTACACA GGAGATACAG AGAAAGTGTT AGTGGCTGGA ATAGAAGAAG AAAAGTAACA ACAGCAGTCA

561   GCTTACGTCT TCAAGATTCT TTTCTTCGCC TGGTTCTTCT TTTTCAATTT CTACGTATTC TTCTTCGTAT TCTGGCAGTA
      CGAATGCAGA AGTTCTAAGA AAAGAAGCGG ACCAAGAAGA AAAAGTTAAA GATGCATAAG AAGAAGCATA AGACCGTCAT

                                                                                      SacI
                                                                                      ~~~~
641   TAGGATCTTG TATCTGTACA TTCTTCATTT TTGAACATAG GTTGCATATG TGCCGCATAT TGATCTGCTT CTTGCTGAGC
      ATCCTAGAAC ATAGACATGT AAGAAGTAAA AACTTGTATC CAACGTATAC ACGGCGTATA ACTAGACGAA GAACGACTCG

      SacI                  HindIII
      ~~                    ~~~~~~
721   TCACATAATA CTTCCATAGG AAGCTTCAGA AGGTAATTAT CCAAGATGTA GCATCAAGAA TCCAATGTTT ACGGGAAAAA
      AGTGTATTAT GAAGGTATCC TTCGAAGTCT TCCATTAATA GGTTCTACAT CGTAGTTCTT AGGTTACAAA TGCCCTTTTT

                    SacI
                    ~~~~~~~
801   CTATGGAAGT ATTATGTGAG CTCAGCAAGA AGCAGATCAA TATGCGGCAC ATATGCAACC TATGTTCAAA AATGAAGAAT
      GATACCTTCA TAATACACTC GAGTCGTTCT TCGTCTAGTT ATACGCCGTG TATACGTTGG ATACAAGTTT TTACTTCTTA

881   GTACAGATAC AAGATCCTAT ACTGCCAGAA TACGAAGAAG AATACGTAGA AATTGAAAAA GAAGAACCAG GCGAAGAAAA
      CATGTCTATG TTCTAGGATA TGACGGTCTT ATGCTTCTTC TTATGCATCT TTAACTTTTT CTTCTTGGTC CGCTTCTTTT

961   GAATCTTGAA GACGTAAGCA CTGACGACAA CAATGAAAAG AAGAAGATAA GGTCGGTGAT TGTGAAAGAG ACATAGAGGA
      CTTAGAACTT CTGCATTCGT GACTGCTGTT GTTACTTTTC TTCTTCTATT CCAGCCACTA ACACTTTCTC TGTATCTCCT

                                  PstI
                                  ~~~~~~~
1041  CACATGTAAG GTGGAAAATG TAAGGGCTGC AGAAGGTAAT TATCCAAGAT GTAGCATCAA GAATCCAATG TTTACGGGAA
      GTGTACATTC CACCTTTTAC ATTCCCGACG TCTTCCATTA ATAGGTTCTA CATCGTAGTT CTTAGGTTAC AAATGCCCTT

                            SacI
                            ~~~~~~
1121  AAACTATGGA AGTATTATGT GAGCTCAGCA AGAAGCAGAT CAATATGCGG CACATATGCA ACCTATGTTC AAAAATGAAG
      TTTGATACCT TCATAATACA CTCGAGTCGT TCTTCGTCTA GTTATACGCC GTGTATACGT TGGATACAAG TTTTTACTTC
```

```
1201  AATGTACAGA TACAAGATCC TATACTGCCA GAATACGAAG AAGAATACGT AGAAATTGAA AAAGAAGAAC CAGGCGAAGA
      TTACATGTCT ATGTTCTAGG ATATGACGGT CTTATGCTTC TTCTTATGCA TCTTTAACTT TTTCTTCTTG GTCCGCTTCT

1281  AAAGAATCTT GAAGACGTAA GCACTGACGA CAACAATGAA AAGAAGAAGA TAAGGTCGGT GATTGTGAAA GAGACATAGA
      TTTCTTAGAA CTTCTGCATT CGTGACTGCT GTTGTTACTT TTCTTCTTCT ATTCCAGCCA CTAACACTTT CTCTGTATCT

1361  GGACACATGT AAGGTGGAAA ATGTAAGGGC GGAAAGTAAC CTTATCACAA AGGAATCTTA TCCCCCACTA CTTATCCTTT
      CCTGTGTACA TTCCACCTTT TACATTCCCG CCTTTCATTG GAATAGTGTT TCCTTAGAAT AGGGGGTGAT GAATAGGAAA

1441  TATATTTTTC CGTGTCATTT TTGCCCTTGA GTTTTCCTAT ATAAGGAACC AAGTTCGGCA TTTGTGAAAA CAAGAAAAAA
      ATATAAAAAG GCACAGTAAA AACGGGAACT CAAAAGGATA TATTCCTTGG TTCAAGCCGT AAACACTTTT GTTCTTTTTT

                                                                                  BamHI
                                                                                  ~~~~~~
1521  TTTGGTGTAA GCTATTTTCT TTGAAGTACT GAGGATACAA CTTCAGAGAA ATTTGTAAGT TTGTGGATCC   Seq. ID No. 7
      AAACCACATT CGATAAAAGA AACTTCATGA CTCCTATGTT GAAGTCTCTT TAAACATTCA AACACCTAGG   Seq. ID No. 8
```

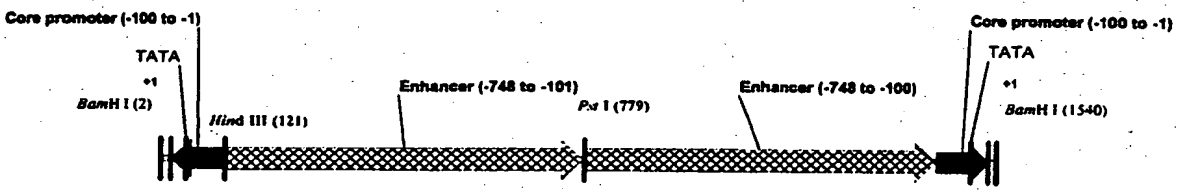
Fig. 9



BDPC with 2 enhancers based on ACT2 promoter
1228 bp

```
      BamHI
      ~~~~~~
   1  GGATCCTTGT TTTCAAAGCG GAGAGGAAAA TATATGAATT TATATAGGCG GGTTTATCTC TTACAACTTT ATTTTCGGCC
      CCTAGGAACA AAAGTTTCGC CTCTCCTTTT ATATACTTAA ATATATCCGC CCAAATAGAG AATGTTGAAA TAAAAGCCGG
 ---------------------------------------------------------------------------------------------
                                                          HindIII
                                                          ~~~~~~~
  81  TTTCAAAAAA ATAATTAAAA TCGACAGACA CGAATCATTT CGACCACAGA AGCTTCAACT ATTTTTATGT ATGCAAGAGT
      AAAGTTTTTT TATTAATTTT AGCTGTCTGT GCTTAGTAAA GCTGGTGTCT TCGAAGTTGA TAAAAATACA TACGTTCTCA
 ---------------------------------------------------------------------------------------------
 161  CAGCATATGT ATAATTGATT CAGAATCGTT TTGACGAGTT CGGATGTAGT AGTAGCCATT ATTTAATGTA CATACTAATC
      GTCGTATACA TATTAACTAA GTCTTAGCAA AACTGCTCAA GCCTACATCA TCATCGGTAA TAAATTACAT GTATGATTAG
 ---------------------------------------------------------------------------------------------
 241  GTGAATAGTG ATATGATGAA ACATTGTATC TTATTGTATA AATATCCATA AACACATCAT GAAAGACACT TTCTTTCACG
      CACTTATCAC TATACTACTT TGTAACATAG AATAACATAT TTATAGGTAT TTGTGTAGTA CTTTCTGTGA AAGAAAGTGC
 ---------------------------------------------------------------------------------------------
 321  GTCTGAATTA ATTATGATAC AATTCTAATA GAAAACGAAT TAAATTACGT TGAATTGTAT GAAATCTAAT TGAACAAGCC
      CAGACTTAAT TAATACTATG TTAAGATTAT CTTTTGCTTA ATTTAATGCA ACTTAACATA CTTTAGATTA ACTTGTTCGG
 ---------------------------------------------------------------------------------------------
 401  AACCACGACG ACGACTAACG TTGCCTGGAT TGACTCGGTT TAAGTTAACC ACTAAAAAAA CGGAGCTGTC ATGTAACACG
      TTGGTGCTGC TGCTGATTGC AACGGACCTA ACTGAGCCAA ATTCAATTGG TGATTTTTTT GCCTCGACAG TACATTGTGC
 ---------------------------------------------------------------------------------------------
 481  CGGATCGAGC AGGTCACAGT CATGAAGCCA TCAAAGCAAA AGAACTAATC CAAGGGCTGA GATGATTAAT TAGTTTAAAA
      GCCTAGCTCG TCCAGTGTCA GTACTTCGGT AGTTTCGTTT TCTTGATTAG GTTCCCGACT CTACTAATTA ATCAAATTTT
 ---------------------------------------------------------------------------------------------
                                                                PstI
                                                                ~~~~~~
 561  ATTAGTTAAC ACGAGGGAAA AGGCTGTCTG ACAGCCAGGT CACGTTATCT TTACCTGCAG CAACTATTTT TATGTATGCA
      TAATCAATTG TGCTCCCTTT TCCGACAGAC TGTCGGTCCA GTGCAATAGA AATGGACGTC GTTGATAAAA ATACATACGT
 ---------------------------------------------------------------------------------------------
 641  AGAGTCAGCA TATGTATAAT TGATTCAGAA TCGTTTTGAC GAGTTCGGAT GTAGTAGTAG CCATTATTTA ATGTACATAC
      TCTCAGTCGT ATACATATTA ACTAAGTCTT AGCAAAACTG CTCAAGCCTA CATCATCATC GGTAATAAAT TACATGTATG
 ---------------------------------------------------------------------------------------------
 721  TAATCGTGAA TAGTGATATG ATGAAACATT GTATCTTATT GTATAAATAT CCATAAACAC ATCATGAAAG ACACTTTCTT
      ATTAGCACTT ATCACTATAC TACTTTGTAA CATAGAATAA CATATTTATA GGTATTTGTG TAGTACTTTC TGTGAAAGAA
 ---------------------------------------------------------------------------------------------
 801  TCACGGTCTG AATTAATTAT GATACAATTC TAATAGAAAA CGAATTAAAT TACGTTGAAT TGTATGAAAT CTAATTGAAC
      AGTGCCAGAC TTAATTAATA CTATGTTAAG ATTATCTTTT GCTTAATTTA ATGCAACTTA ACATACTTTA GATTAACTTG
 ---------------------------------------------------------------------------------------------
 881  AAGCCAACCA CGACGACGAC TAACGTTGCC TGGATTGACT CGGTTTAAGT TAACCACTAA AAAAACGGAG CTGTCATGTA
      TTCGGTTGGT GCTGCTGCTG ATTGCAACGG ACCTAACTGA GCCAAATTCA ATTGGTGATT TTTTTGCCTC GACAGTACAT
 ---------------------------------------------------------------------------------------------
 961  ACACGCGGAT CGAGCAGGTC ACAGTCATGA AGCCATCAAA GCAAAAGAAC TAATCCAAGG GCTGAGATGA TTAATTAGTT
      TGTGCGCCTA GCTCGTCCAG TGTCAGTACT TCGGTAGTTT CGTTTTCTTG ATTAGGTTCC CGACTCTACT AATTAATCAA
 ---------------------------------------------------------------------------------------------
1041  TAAAAATTAG TTAACACGAG GGAAAAGGCT GTCTGACAGC CAGGTCACGT TATCTTTACC TGTGGTCGAA ATGATTCGTG
      ATTTTTAATC AATTGTGCTC CCTTTTCCGA CAGACTGTCG GTCCAGTGCA ATAGAAATGG ACACCAGCTT TACTAAGCAC
 ---------------------------------------------------------------------------------------------
1121  TCTGTCGATT TTAATTATTT TTTTGAAAGG CCGAAAATAA AGTTGTAAGA GATAAACCCG CCTATATAAA TTCATATATT
      AGACAGCTAA AATTAATAAA AAAACTTTCC GGCTTTTATT TCAACATTCT CTATTTGGGC GGATATATTT AAGTATATAA
 ---------------------------------------------------------------------------------------------
                                BamHI
                                ~~~~~~
1201  TTCCTCTCCG CTTTGAAAAC AAGGATCC  Seq. ID No. 9
      AAGGAGAGGC GAAACTTTTG TTCCTAGG  Seq. ID No. 10
 ---------------------------------------------------------------------------------------------
```

Fig. 11



BDPC with 2 enhancers based on PR1b promoter of tobacco

1544 bp

BamHI

```
  1  GGATCCTTTT GGGTTTTGGT GAGAAACAAG GAATAGTATG GATGGGTTTT AATAGGGAAT AAGAGTTGAA AAGTCTGCAA
     CCTAGGAAAA CCCAAAACCA CTCTTTGTTC CTTATCATAC CTACCCAAAA TTATCCCTTA TTCTCAACTT TTCAGACGTT
```

HindIII

```
 81  TTTGTAAAAG AAAAAAATTG GAAAGTCACA TGTTAGCAGA AGCTTCAGAC TCATTAACTT AAAAGAAGAT ATAGACTCAT
     AAACATTTTC TTTTTTTAAC CTTTCAGTGT ACAATCGTCT TCGAAGTCTG AGTAATTGAA TTTTCTTCTA TATCTGAGTA

161  TAACTTAAAA GAAGATATAG ATTCCAACAC AAGTTCAAAA TTCATAAACG TCAATCTTGG CTAAATTTCT GAACATCAAT
     ATTGAATTTT CTTCTATATC TAAGGTTGTG TTCAAGTTTT AAGTATTTGC AGTTAGAACC GATTTAAAGA CTTGTAGTTA

241  GCATTCCTTT AAAATATAGA TAATAAGTTA GGATGTTGTC ACTTTCTTAA AGCATATTCC GACTGAGTCT GGTAGAATCT
     CGTAAGGAAA TTTTATATCT ATTATTCAAT CCTACAACAG TGAAAGAATT TCGTATAAGG CTGACTCAGA CCATCTTAGA

321  CATAAACTTT AGGCCTTATC TCTTCAATTA GGCAATTACT TACCTCCGCT CTACTTTAAG AAAATTCAAT GGAGTACACC
     GTATTTGAAA TCCGGAATAG AGAAGTTAAT CCGTTAATGA ATGGAGGCGA GATGAAATTC TTTTAAGTTA CCTCATGTGG

401  ATTATTAAGT TCATATAAAA ATAAAATTAT ATTAATTCTG TCTCTTGTTG GTTCGCTCTA TCTTTTTCTG TTTTCCTGCT
     TAATAATTCA AGTATATTTT TATTTTAATA TAATTAAGAC AGAGAACAAC CAAGCGAGAT AGAAAAAGAC AAAAGGACGA

481  TCAACCATAA CATATACAAG AACTACATTT TCCAAGCTAG ATATATCTAA CATGACTGAC TTTGTAAATT TCTTTTGCCA
     AGTTGGTATT GTATATGTTC TTGATGTAAA AGGTTCGATC TATATAGATT GTACTGACTG AAACATTTAA AGAAAACGGT

561  AGTTAAAGAA AAAAAATGAT GTTATCCAAA TAATAAAGAG AAAGAGCCCT AATGAAAAAA ATGATTTACT ATTAGAGTTG
     TCAATTTCTT TTTTTTACTA CAATAGGTTT ATTATTTCTC TTTCTCGGGA TTACTTTTTT TACTAAATGA TAATCTCAAC

641  TTCAGCTAAT CACATCAATT ATGGTTTTCA TCAAGTATGA CTAATGGCGG CTCTTATCTC ACGTGATGTG ACATTGAAAT
     AAGTCGATTA GTGTAGTTAA TACCAAAAGT AGTTCATACT GATTACCGCC GAGAATAGAG TGCACTACAC TGTAACTTTA
```

PstI

```
721  TCTTTGACTT TAACACTAAT GTCATATGCT TTCAAATTAA TAATCCGATA AAGCTGCAGA CTCATTAACT TAAAAGAAGA
     AGAAACTGAA ATTGTGATTA CAGTATACGA AAGTTTAATT ATTAGGCTAT TTCGACGTCT GAGTAATTGA ATTTTCTTCT

801  TATAGACTCA TTAACTTAAA AGAAGATATA GATTCCAACA CAAGTTCAAA ATTCATAAAC GTCAATCTTG GCTAAATTTC
     ATATCTGAGT AATTGAATTT TCTTCTATAT CTAAGGTTGT GTTCAAGTTT TAAGTATTTG CAGTTAGAAC CGATTTAAAG

881  TGAACATCAA TGCATTCCTT TAAAATATAG ATAATAAGTT AGGATGTTGT CACTTTCTTA AAGCATATTC CGACTGAGTC
     ACTTGTAGTT ACGTAAGGAA ATTTTATATC TATTATTCAA TCCTACAACA GTGAAAGAAT TTCGTATAAG GCTGACTCAG

961  TGGTAGAATC TCATAAACTT TAGGCCTTAT CTCTTCAATT AGGCAATTAC TTACCTCCGC TCTACTTTAA GAAAATTCAA
     ACCATCTTAG AGTATTTGAA ATCCGGAATA GAGAAGTTAA TCCGTTAATG AATGGAGGCG AGATGAAATT CTTTTAAGTT

1041 TGGAGTACAC CATTATTAAG TTCATATAAA AATAAAATTA TATTAATTCT GTCTCTTGTT GGTTCGCTCT ATCTTTTTCT
     ACCTCATGTG GTAATAATTC AAGTATATTT TTATTTTAAT ATAATTAAGA CAGAGAACAA CCAAGCGAGA TAGAAAAAGA

1121 GTTTTCCTGC TTCAACCATA ACATATACAA GAACTACATT TTCCAAGCTA GATATATCTA ACATGACTGA CTTTGTAAAT
     CAAAAGGACG AAGTTGGTAT TGTATATGTT CTTGATGTAA AAGGTTCGAT CTATATAGAT TGTACTGACT GAAACATTTA

1201 TTCTTTTGCC AAGTTAAAGA AAAAAAATGA TGTTATCCAA ATAATAAAGA GAAAGAGCCC TAATGAAAAA AATGATTTAC
     AAGAAAACGG TTCAATTTCT TTTTTTTACT ACAATAGGTT TATTATTTCT CTTTCTCGGG ATTACTTTTT TTACTAAATG

1281 TATTAGAGTT GTTCAGCTAA TCACATCAAT TATGGTTTTC ATCAAGTATG ACTAATGGCG GCTCTTATCT CACGTGATGT
     ATAATCTCAA CAAGTCGATT AGTGTAGTTA ATACCAAAAG TAGTTCATAC TGATTACCGC CGAGAATAGA GTGCACTACA
```

```
1361  GACATTGAAA TTCTTTGACT TTAACACTAA TGTCATATGC TTTCAAATTA ATAATCCGAT AAAGTCTGCT AACATGTGAC
      CTGTAACTTT AAGAAACTGA AATTGTGATT ACAGTATACG AAAGTTTAAT TATTAGGCTA TTTCAGACGA TTGTACACTG

1441  TTTCCAATTT TTTTCTTTTA CAAATTGCAG ACTTTTCAAC TCTTATTCCC TATTAAAACC CATCCATACT ATTCCTTGTT
      AAAGGTTAAA AAAAGAAAAT GTTTAACGTC TGAAAAGTTG AGAATAAGGG ATAATTTTGG GTAGGTATGA TAAGGAACAA

                        BamHI
                        ~~~~~~~
1521  TCTCACCAAA ACCCAAAAGG ATCC   Seq. ID No. 11
      AGAGTGGTTT TGGGTTTTCC TAGG   Seq. ID No. 12
```

Figure 13. Physical Map of T-DNA Region of Binary Vectors Containing a BDPC

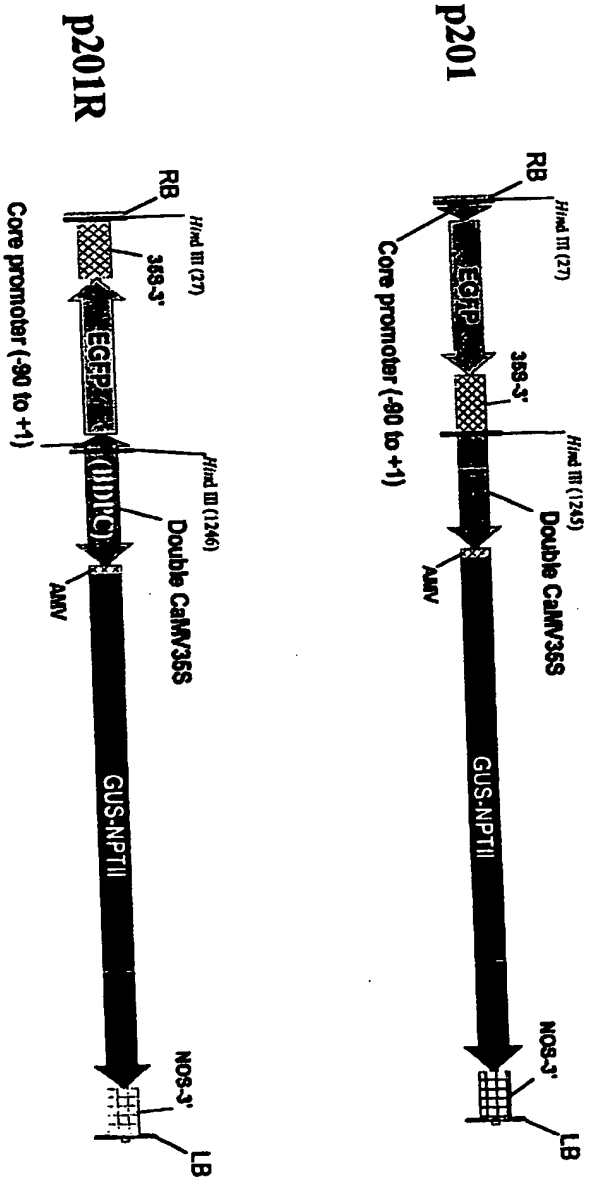


Figure 14. Transient GFP Expression in Grape SE (*Vitis vinifera* cv. Thompson Seedless) after Transformation Using Binary Vectors p201 and p201R

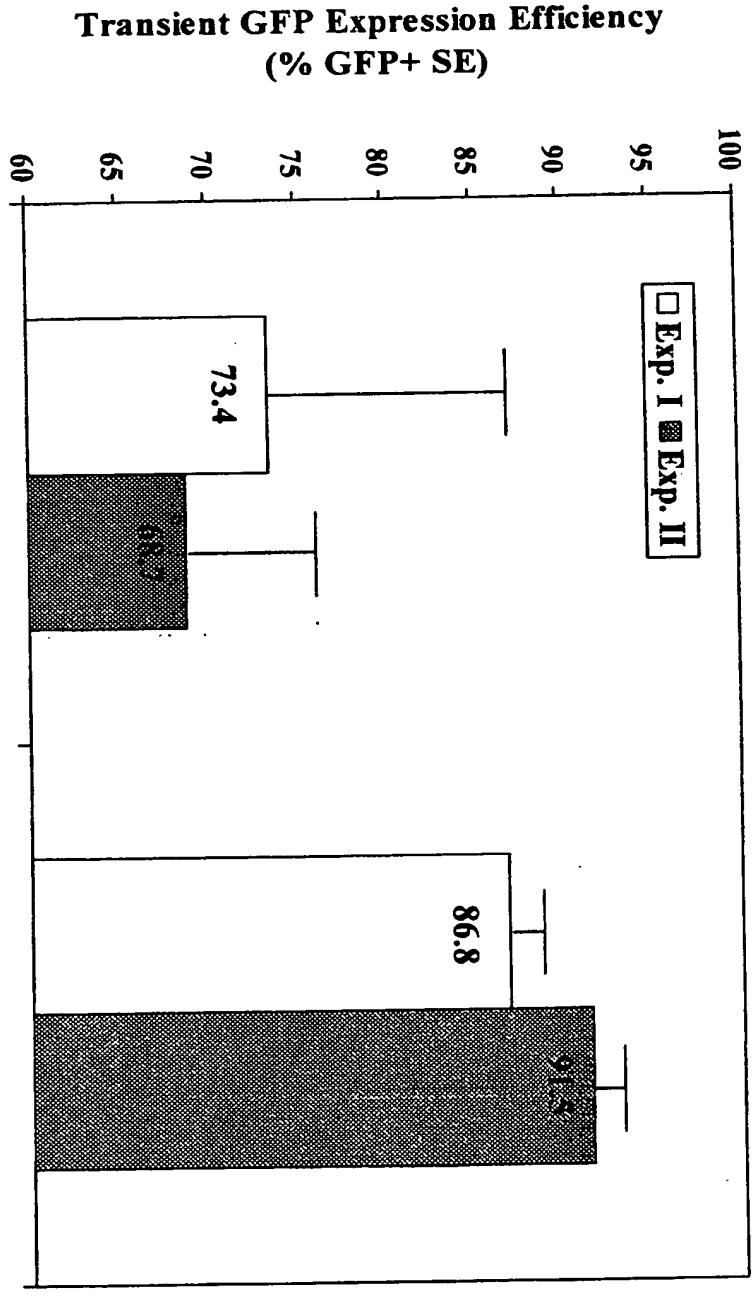


Figure 15. Transient GFP Expression Efficiency of Grape SE (*V. vinifera* cv. Thompson Seedless) after Transformation Using Binary Vectors p201 And p201R

Figure 16. Analysis of GUS Activity in Grape SE (*V. vinifera* cv. Thompson Seedless) after Transformation Using Binary Vectors p201 and p201R

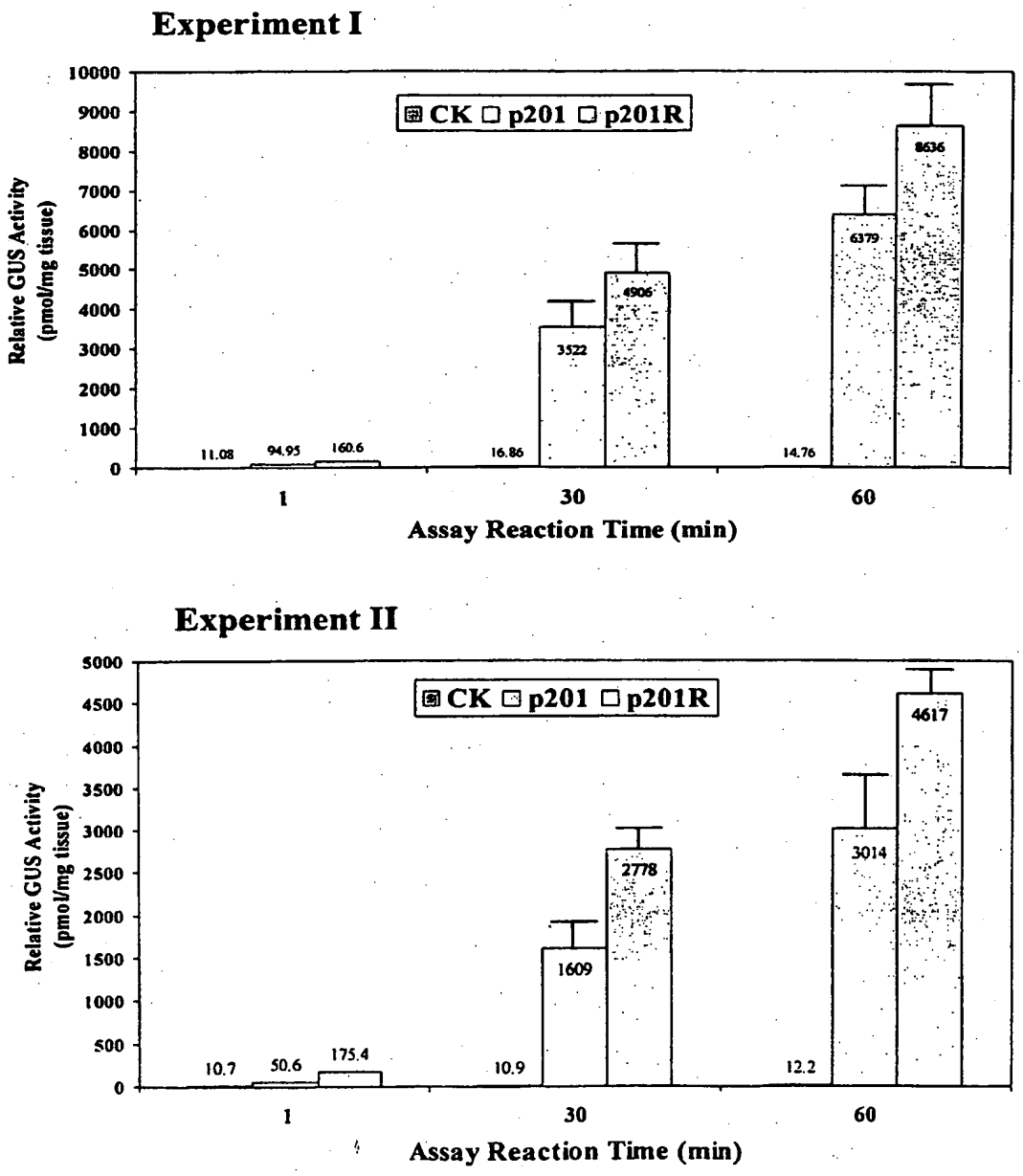


Figure 17. GFP Expression in SE (A) and Leaf Tissues (B) of Transgenic Grape (*V. vinifera* cv. Thompson Seedless) Containing the T-DNA of p201R

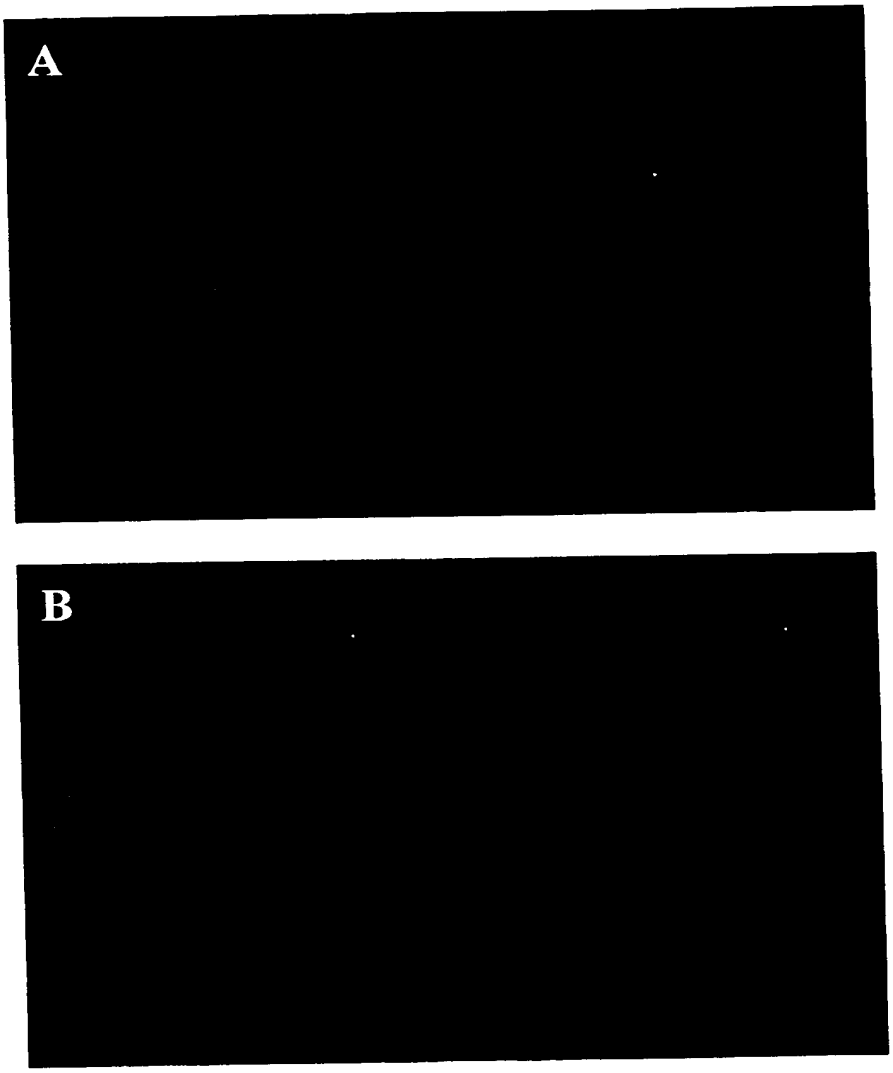
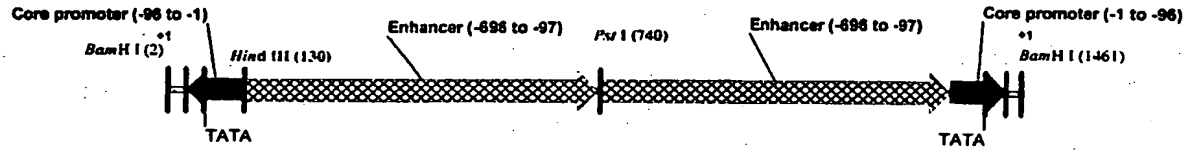


Fig. 18



BDPC with 2 enhancers based on At UBQ1 promoter

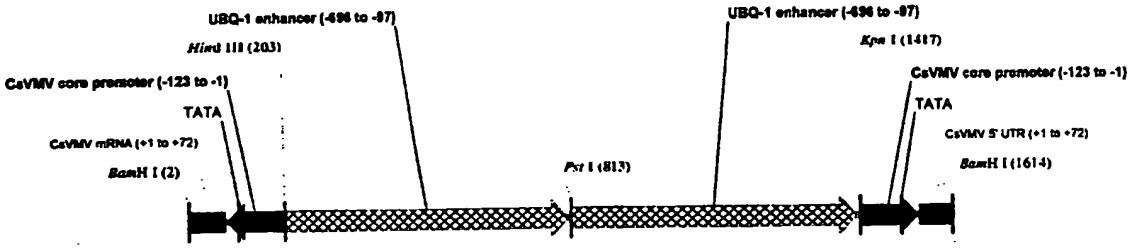
1465 bp

Fig. 19

```
      BamHI
      ~~~~~~
  1   GGATCCCTTT TGTGTTTCGT CTTCTCTCAC GTAGAAACCC TAAACAAGGA GGAGGCGGGT TTATATATGT CAATGTACGC
      CCTAGGGAAA ACACAAAGCA GAAGAGAGTG CATCTTTGGG ATTTGTTCCT CCTCCGCCCA AATATATACA GTTACATGCG
-----------------------------------------------------------------------------------------
                                                          HindIII
                                                          ~~~~~~~
 81   GTCTAGGGTT TTGCTAATAT TGGGCTAGGT TACAGGCCTT TACCACAAAA GCTTAGTTGA TAAAATATTT TTATTTGGTT
      CAGATCCCAA AACGATTATA ACCCGATCCA ATGTCCGGAA ATGGTGTTTT CGAATCAACT ATTTTATAAA AATAAACCAA
-----------------------------------------------------------------------------------------
161   GTAATTTTGT AATATCCCGG GATATTTCAC AAATTGAACA TAGACTACAG AATTTTAGAA AACAAACTTT CTCTCTCTTA
      CATTAAAACA TTATAGGGCC CTATAAAGTG TTTAACTTGT ATCTGATGTC TTAAAATCTT TTGTTTGAAA GAGAGAGAAT
-----------------------------------------------------------------------------------------
241   TCTCACCTTT ATCTTTTAGA GAGAAAAAGT TCGATTTCCG GTTGACCGGA ATGTATCTTT GTTTTTTTTG TTTTGTAACA
      AGAGTGGAAA TAGAAAATCT CTCTTTTTCA AGCTAAAGGC CAACTGGCCT TACATAGAAA CAAAAAAAAC AAAACATTGT
-----------------------------------------------------------------------------------------
321   TATTTCGTTT TCCGATTTAG ATCGGATCTC CTTTTCCGTT TTGTCGGACC TTCTTCCGGT TTATCCGGAT CTAATAATAT
      ATAAAGCAAA AGGCTAAATC TAGCCTAGAG GAAAAGGCAA AACAGCCTGG AAGAAGGCCA AATAGGCCTA GATTATTATA
-----------------------------------------------------------------------------------------
401   CCATCTTAGA CTTAGCTAAG TTTGGATCTG TTTTTTGGTT AGCTCTTGTC AATCGCCTCA TCATCAGCAA GAAGGTGAAA
      GGTAGAATCT GAATCGATTC AAACCTAGAC AAAAAACCAA TCGAGAACAG TTAGCGGAGT AGTAGTCGTT CTTCCACTTT
-----------------------------------------------------------------------------------------
481   TTTTTGACAA ATAAATCTTA GAATCATGTA GTGTCTTTGG ACCTTGGGAA TGATAGAAAC GATTTGTTAT AGCTACTCTA
      AAAAACTGTT TATTTAGAAT CTTAGTACAT CACAGAAACC TGGAACCCTT ACTATCTTTG CTAAACAATA TCGATGAGAT
-----------------------------------------------------------------------------------------
561   TGTATCAGAC CCTGACCAAG ATCCAACAAT CTCATAGGTT TTGTGCATAT GAAACCTTCG ACTAACGAGA AGTGGTCTTT
      ACATAGTCTG GGACTGGTTC TAGGTTGTTA GAGTATCCAA AACACGTATA CTTTGGAAGC TGATTGCTCT TCACCAGAAA
-----------------------------------------------------------------------------------------
641   TAATGAGAGA GATATCTAAA ATGTTATCTT AAAAGCCCAC TCAAATCTCA AGGCATAAGG TAGAAATGCA AATTTGGAAA
      ATTACTCTCT CTATAGATTT TACAATAGAA TTTTCGGGTG AGTTTAGAGT TCCGTATTCC ATCTTTACGT TTAAACCTTT
-----------------------------------------------------------------------------------------
                      PstI
                      ~~~~~~
721   GTGGGCTGGG CCTTCTGCAG TTGATAAAAT ATTTTTATTT GGTTGTAATT TTGTAATATC CCGGGATATT TCACAAATTG
      CACCCGACCC GGAAGACGTC AACTATTTTA TAAAAATAAA CCAACATTAA AACATTATAG GGCCCTATAA AGTGTTTAAC
-----------------------------------------------------------------------------------------
801   AACATAGACT ACAGAATTTT AGAAAACAAA CTTTCTCTCT CTTATCTCAC CTTTATCTTT TAGAGAGAAA AAGTTCGATT
      TTGTATCTGA TGTCTTAAAA TCTTTTGTTT GAAAGAGAGA GAATAGAGTG GAAATAGAAA ATCTCTCTTT TTCAAGCTAA
-----------------------------------------------------------------------------------------
881   TCCGGTTGAC CGGAATGTAT CTTTGTTTTT TTTGTTTTGT AACATATTTC GTTTTCCGAT TTAGATCGGA TCTCCTTTTC
      AGGCCAACTG GCCTTACATA GAAACAAAAA AAACAAAACA TTGTATAAAG CAAAAGGCTA AATCTAGCCT AGAGGAAAAG
-----------------------------------------------------------------------------------------
961   CGTTTTGTCG GACCTTCTTC CGGTTTATCC GGATCTAATA ATATCCATCT TAGACTTAGC TAAGTTTGGA TCTGTTTTTT
      GCAAAACAGC CTGGAAGAAG GCCAAATAGG CCTAGATTAT TATAGGTAGA ATCTGAATCG ATTCAAACCT AGACAAAAAA
-----------------------------------------------------------------------------------------
1041  GGTTAGCTCT TGTCAATCGC CTCATCATCA GCAAGAAGGT GAAATTTTTG ACAAATAAAT CTTAGAATCA TGTAGTGTCT
      CCAATCGAGA ACAGTTAGCG GAGTAGTAGT CGTTCTTCCA CTTTAAAAAC TGTTTATTTA GAATCTTAGT ACATCACAGA
-----------------------------------------------------------------------------------------
1121  TTGGACCTTG GGAATGATAG AAACGATTTG TTATAGCTAC TCTATGTATC AGACCCTGAC CAAGATCCAA CAATCTCATA
      AACCTGGAAC CCTTACTATC TTTGCTAAAC AATATCGATG AGATACATAG TCTGGGACTG GTTCTAGGTT GTTAGAGTAT
-----------------------------------------------------------------------------------------
1201  GGTTTTGTGC ATATGAAACC TTCGACTAAC GAGAAGTGGT CTTTTAATGA GAGAGATATC TAAAATGTTA TCTTAAAAGC
      CCAAAACACG TATACTTTGG AAGCTGATTG CTCTTCACCA GAAAATTACT CTCTCTATAG ATTTTACAAT AGAATTTTCG
-----------------------------------------------------------------------------------------
1281  CCACTCAAAT CTCAAGGCAT AAGGTAGAAA TGCAAATTTG GAAAGTGGGC TGGGCCTTTT GTGGTAAAGG CCTGTAACCT
      GGTGAGTTTA GAGTTCCGTA TTCCATCTTT ACGTTTAAAC CTTTCACCCG ACCCGGAAAA CACCATTTCC GGACATTGGA
-----------------------------------------------------------------------------------------
```

```
1361  AGCCCAATAT TAGCAAAACC CTAGACGCGT ACATTGACAT ATATAAACCC GCCTCCTCCT TGTTTAGGGT TTCTACGTGA
      TCGGGTTATA ATCGTTTTGG GATCTGCGCA TGTAACTGTA TATATTTGGG CGGAGGAGGA ACAAATCCCA AAGATGCACT
 ----------------------------------------------------------------------------------------------

                            BamHI
                           ~~~~~~~
1441  GAGAAGACGA AACACAAAAG GATCC  Seq. ID No. 13
      CTCTTCTGCT TTGTGTTTTC CTAGG  Seq. ID No. 14
 ----------------------------------------------------------------------------------------------
```

Fig. 20



Heterologous BDPC with 2 UBQ-1 enhancers and 2 CsVMV core promoters

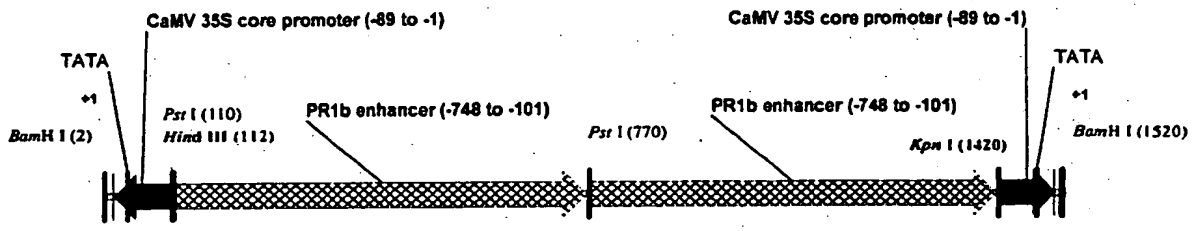
1618 bp

Fig. 21

```
      BamHI
      ~~~~~~
   1  GGATCCACAA ACTTACAAAT TTCTCTGAAG TTGTATCCTC AGTACTTCAA AGAAAATAGC TTACACCAAA TTTTTTCTTG
      CCTAGGTGTT TGAATGTTTA AAGAGACTTC AACATAGGAG TCATGAAGTT TCTTTTATCG AATGTGGTTT AAAAAAGAAC

  81  TTTTCACAAA TGCCGAACTT GGTTCCTTAT ATAGGAAAAC TCAAGGGCAA AAATGACACG GAAAAATATA AAAGGATAAG
      AAAAGTGTTT ACGGCTTGAA CCAAGGAATA TATCCTTTTG AGTTCCCGTT TTTACTGTGC CTTTTTATAT TTTCCTATTC

                                                  HindIII
                                                  ~~~~~~
 161  TAGTGGGGGA TAAGATTCCT TTGTGATAAG GTTACTTTCC GAAGCTTAGT TGATAAAATA TTTTTATTTG GTTGTAATTT
      ATCACCCCCT ATTCTAAGGA AACACTATTC CAATGAAAGG CTTCGAATCA ACTATTTTAT AAAAATAAAC CAACATTAAA

 241  TGTAATATCC CGGGATATTT CACAAATTGA ACATAGACTA CAGAATTTTA GAAAACAAAC TTTCTCTCTC TTATCTCACC
      ACATTATAGG GCCCTATAAA GTGTTTAACT TGTATCTGAT GTCTTAAAAT CTTTTGTTTG AAAGAGAGAG AATAGAGTGG

 321  TTTATCTTTT AGAGAGAAAA AGTTCGATTT CCGGTTGACC GGAATGTATC TTTGTTTTTT TTGTTTTGTA ACATATTTCG
      AAATAGAAAA TCTCTCTTTT TCAAGCTAAA GGCCAACTGG CCTTACATAG AAACAAAAAA AACAAAACAT TGTATAAAGC

 401  TTTTCCGATT TAGATCGGAT CTCCTTTTCC GTTTTGTCGG ACCTTCTTCC GGTTTATCCG GATCTAATAA TATCCATCTT
      AAAAGGCTAA ATCTAGCCTA GAGGAAAAGG CAAAACAGCC TGGAAGAAGG CCAAATAGGC CTAGATTATT ATAGGTAGAA

 481  AGACTTAGCT AAGTTTGGAT CTGTTTTTTG GTTAGCTCTT GTCAATCGCC TCATCATCAG CAAGAAGGTG AAATTTTTGA
      TCTGAATCGA TTCAAACCTA GACAAAAAAC CAATCGAGAA CAGTTAGCGG AGTAGTAGTC GTTCTTCCAC TTTAAAAACT

 561  CAAATAAATC TTAGAATCAT GTAGTGTCTT TGGACCTTGG GAATGATAGA AACGATTTGT TATAGCTACT CTATGTATCA
      GTTTATTTAG AATCTTAGTA CATCACAGAA ACCTGGAACC CTTACTATCT TTGCTAAACA ATATCGATGA GATACATAGT

 641  GACCCTGACC AAGATCCAAC AATCTCATAG GTTTTGTGCA TATGAAACCT TCGACTAACG AGAAGTGGTC TTTTAATGAG
      CTGGGACTGG TTCTAGGTTG TTAGAGTATC CAAAACACGT ATACTTTGGA AGCTGATTGC TCTTCACCAG AAAATTACTC

 721  AGAGATATCT AAAATGTTAT CTTAAAAGCC CACTCAAATC TCAAGGCATA AGGTAGAAAT GCAAATTTGG AAAGTGGGCT
      TCTCTATAGA TTTTACAATA GAATTTTCGG GTGAGTTTAG AGTTCCGTAT TCCATCTTTA CGTTTAAACC TTTCACCCGA

            PstI
            ~~~~~~~
 801  GGGCCTTCTG CAGTTGATAA AATATTTTTA TTTGGTTGTA ATTTTGTAAT ATCCCGGGAT ATTTCACAAA TTGAACATAG
      CCCGGAAGAC GTCAACTATT TTATAAAAAT AAACCAACAT TAAAACATTA TAGGGCCCTA TAAAGTGTTT AACTTGTATC

 881  ACTACAGAAT TTTAGAAAAC AAACTTTCTC TCTCTTATCT CACCTTTATC TTTTAGAGAG AAAAAGTTCG ATTTCCGGTT
      TGATGTCTTA AAATCTTTTG TTTGAAAGAG AGAGAATAGA GTGGAAATAG AAAATCTCTC TTTTTCAAGC TAAAGGCCAA

 961  GACCGGAATG TATCTTTGTT TTTTTTGTTT TGTAACATAT TTCGTTTTCC GATTTAGATC GGATCTCCTT TTCCGTTTTG
      CTGGCCTTAC ATAGAAACAA AAAAAACAAA ACATTGTATA AAGCAAAAGG CTAAATCTAG CCTAGAGGAA AAGGCAAAAC

1041  TCGGACCTTC TTCCGGTTTA TCCGGATCTA ATAATATCCA TCTTAGACTT AGCTAAGTTT GGATCTGTTT TTTGGTTAGC
      AGCCTGGAAG AAGGCCAAAT AGGCCTAGAT TATTATAGGT AGAATCTGAA TCGATTCAAA CCTAGACAAA AAACCAATCG

1121  TCTTGTCAAT CGCCTCATCA TCAGCAAGAA GGTGAAATTT TTGACAAATA AATCTTAGAA TCATGTAGTG TCTTTGGACC
      AGAACAGTTA GCGGAGTAGT AGTCGTTCTT CCACTTTAAA AACTGTTTAT TTAGAATCTT AGTACATCAC AGAAACCTGG

1201  TTGGGAATGA TAGAAACGAT TTGTTATAGC TACTCTATGT ATCAGACCCT GACCAAGATC CAACAATCTC ATAGGTTTTG
      AACCCTTACT ATCTTTGCTA AACAATATCG ATGAGATACA TAGTCTGGGA CTGGTTCTAG GTTGTTAGAG TATCCAAAAC

1281  TGCATATGAA ACCTTCGACT AACGAGAAGT GGTCTTTTAA TGAGAGAGAT ATCTAAAATG TTATCTTAAA AGCCCACTCA
      ACGTATACTT TGGAAGCTGA TTGCTCTTCA CCAGAAAATT ACTCTCTCTA TAGATTTTAC AATAGAATTT TCGGGTGAGT
```

```
                                                                    KpnI
                                                                    ~~~~~~
1361  AATCTCAAGG CATAAGGTAG AAATGCAAAT TTGGAAAGTG GGCTGGGCCT TGGTACCCGG AAAGTAACCT TATCACAAAG
      TTAGAGTTCC GTATTCCATC TTTACGTTTA AACCTTTCAC CCGACCCGGA ACCATGGGCC TTTCATTGGA ATAGTGTTTC
-------------------------------------------------------------------------------------------
1441  GAATCTTATC CCCCACTACT TATCCTTTTA TATTTTTCCG TGTCATTTTT GCCCTTGAGT TTTCCTATAT AAGGAACCAA
      CTTAGAATAG GGGGTGATGA ATAGGAAAAT ATAAAAAGGC ACAGTAAAAA CGGGAACTCA AAAGGATATA TTCCTTGGTT
-------------------------------------------------------------------------------------------
1521  GTTCGGCATT TGTGAAAACA AGAAAAAATT TGGTGTAAGC TATTTTCTTT GAAGTACTGA GGATACAACT TCAGAGAAAT
      CAAGCCGTAA ACACTTTTGT TCTTTTTTAA ACCACATTCG ATAAAAGAAA CTTCATGACT CCTATGTTGA AGTCTCTTTA
-------------------------------------------------------------------------------------------
                  BamHI
                  ~~~~~~
1601  TTGTAAGTTT GTGGATCC  Seq. ID No. 15
      AACATTCAAA CACCTAGG  Seq. ID No. 16
-------------------------------------------------------------------------------------------
```

Fig. 22



Heterologous BDPC with 2 PR1b enhancers and 2 CaMV 35S core promoters

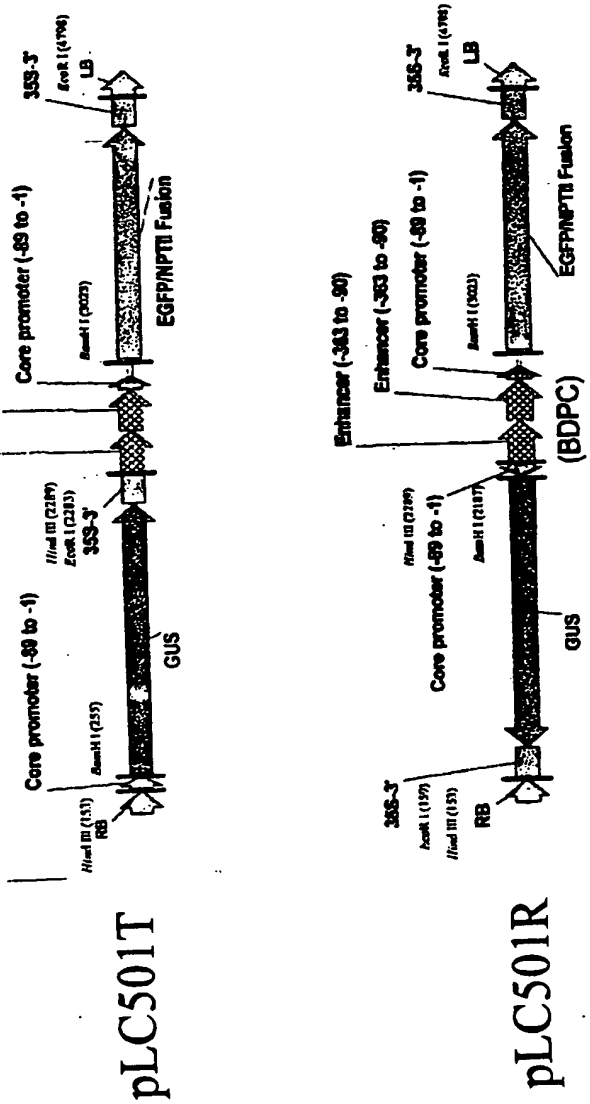
1524 bp

Fig. 23

```
      BamHI
      ~~~~~~
   1  GGATCCAGCG TGTCCTCTCC AAATGAAATG AACTTCCTTA TATAGAGGAA GGGTCTTGCG AAGGATAGTG GGATTGTGCG
      CCTAGGTCGC ACAGGAGAGG TTTACTTTAC TTGAAGGAAT ATATCTCCTT CCCAGAACGC TTCCTATCAC CCTAACACGC
-------------------------------------------------------------------------------------------

                                  PstI  HindIII
                                  ~~~~~~~~~~~~
  81  TCATCCCTTA CGTCAGTGGA GATACTGCAG AAGCTTCAGA CTCATTAACT TAAAAGAAGA TATAGACTCA TTAACTTAAA
      AGTAGGGAAT GCAGTCACCT CTATGACGTC TTCGAAGTCT GAGTAATTGA ATTTTCTTCT ATATCTGAGT AATTGAATTT
-------------------------------------------------------------------------------------------

 161  AGAAGATATA GATTCCAACA CAAGTTCAAA ATTCATAAAC GTCAATCTTG GCTAAATTTC TGAACATCAA TGCATTCCTT
      TCTTCTATAT CTAAGGTTGT GTTCAAGTTT TAAGTATTTG CAGTTAGAAC CGATTTAAAG ACTTGTAGTT ACGTAAGGAA
-------------------------------------------------------------------------------------------

 241  TAAAATATAG ATAATAAGTT AGGATGTTGT CACTTTCTTA AAGCATATTC CGACTGAGTC TGGTAGAATC TCATAAACTT
      ATTTTATATC TATTATTCAA TCCTACAACA GTGAAAGAAT TTCGTATAAG GCTGACTCAG ACCATCTTAG AGTATTTGAA
-------------------------------------------------------------------------------------------

 321  TAGGCCTTAT CTCTTCAATT AGGCAATTAC TTACCTCCGC TCTACTTTAA GAAAATTCAA TGGAGTACAC CATTATTAAG
      ATCCGGAATA GAGAAGTTAA TCCGTTAATG AATGGAGGCG AGATGAAATT CTTTTAAGTT ACCTCATGTG GTAATAATTC
-------------------------------------------------------------------------------------------

 401  TTCATATAAA AATAAAATTA TATTAATTCT GTCTCTTGTT GGTTCGCTCT ATCTTTTTCT GTTTTCCTGC TTCAACCATA
      AAGTATATTT TTATTTTAAT ATAATTAAGA CAGAGAACAA CCAAGCGAGA TAGAAAAAGA CAAAAGGACG AAGTTGGTAT
-------------------------------------------------------------------------------------------

 481  ACATATACAA GAACTACATT TTCCAAGCTA GATATATCTA ACATGACTGA CTTTGTAAAT TTCTTTTGCC AAGTTAAAGA
      TGTATATGTT CTTGATGTAA AAGGTTCGAT CTATATAGAT TGTACTGACT GAAACATTTA AAGAAAACGG TTCAATTTCT
-------------------------------------------------------------------------------------------

 561  AAAAAAATGA TGTTATCCAA ATAATAAAGA GAAAGAGCCC TAATGAAAAA AATGATTTAC TATTAGAGTT GTTCAGCTAA
      TTTTTTTACT ACAATAGGTT TATTATTTCT CTTTCTCGGG ATTACTTTTT TTACTAAATG ATAATCTCAA CAAGTCGATT
-------------------------------------------------------------------------------------------

 641  TCACATCAAT TATGGTTTTC ATCAAGTATG ACTAATGGCG GCTCTTATCT CACGTGATGT GACATTGAAA TTCTTTGACT
      AGTGTAGTTA ATACCAAAAG TAGTTCATAC TGATTACCGC CGAGAATAGA GTGCACTACA CTGTAACTTT AAGAAACTGA
-------------------------------------------------------------------------------------------

                                                      PstI
                                                      ~~~~~~
 721  TTAACACTAA TGTCATATGC TTTCAAATTA ATAATCCGAT AAAGCTGCAG ACTCATTAAC TTAAAAGAAG ATATAGACTC
      AATTGTGATT ACAGTATACG AAAGTTTAAT TATTAGGCTA TTTCGACGTC TGAGTAATTG AATTTTCTTC TATATCTGAG
-------------------------------------------------------------------------------------------

 801  ATTAACTTAA AAGAAGATAT AGATTCCAAC ACAAGTTCAA AATTCATAAA CGTCAATCTT GGCTAAATTT CTGAACATCA
      TAATTGAATT TTCTTCTATA TCTAAGGTTG TGTTCAAGTT TTAAGTATTT GCAGTTAGAA CCGATTTAAA GACTTGTAGT
-------------------------------------------------------------------------------------------

 881  ATGCATTCCT TTAAAATATA GATAATAAGT TAGGATGTTG TCACTTTCTT AAAGCATATT CCGACTGAGT CTGGTAGAAT
      TACGTAAGGA AATTTTATAT CTATTATTCA ATCCTACAAC AGTGAAAGAA TTTCGTATAA GGCTGACTCA GACCATCTTA
-------------------------------------------------------------------------------------------

 961  CTCATAAACT TTAGGCCTTA TCTCTTCAAT TAGGCAATTA CTTACCTCCG CTCTACTTTA AGAAAATTCA ATGGAGTACA
      GAGTATTTGA AATCCGGAAT AGAGAAGTTA ATCCGTTAAT GAATGGAGGC GAGATGAAAT TCTTTTAAGT TACCTCATGT
-------------------------------------------------------------------------------------------

1041  CCATTATTAA GTTCATATAA AAATAAAATT ATATTAATTC TGTCTCTTGT TGGTTCGCTC TATCTTTTTC TGTTTTCCTG
      GGTAATAATT CAAGTATATT TTTATTTTAA TATAATTAAG ACAGAGAACA ACCAAGCGAG ATAGAAAAAG ACAAAAGGAC
-------------------------------------------------------------------------------------------

1121  CTTCAACCAT AACATATACA AGAACTACAT TTTCCAAGCT AGATATATCT AACATGACTG ACTTTGTAAA TTTCTTTTGC
      GAAGTTGGTA TTGTATATGT TCTTGATGTA AAAGGTTCGA TCTATATAGA TTGTACTGAC TGAAACATTT AAAGAAAACG
-------------------------------------------------------------------------------------------

1201  CAAGTTAAAG AAAAAAAATG ATGTTATCCA AATAATAAAG AGAAAGAGCC CTAATGAAAA AAATGATTTA CTATTAGAGT
      GTTCAATTTC TTTTTTTTAC TACAATAGGT TTATTATTTC TCTTTCTCGG GATTACTTTT TTTACTAAAT GATAATCTCA
-------------------------------------------------------------------------------------------

1281  TGTTCAGCTA ATCACATCAA TTATGGTTTT CATCAAGTAT GACTAATGGC GGCTCTTATC TCACGTGATG TGACATTGAA
      ACAAGTCGAT TAGTGTAGTT AATACCAAAA GTAGTTCATA CTGATTACCG CCGAGAATAG AGTGCACTAC ACTGTAACTT
-------------------------------------------------------------------------------------------
```

```
                                                                    KpnI
                                                                    ~~~~~~
1361  ATTCTTTGAC TTTAACACTA ATGTCATATG CTTTCAAATT AATAATCCGA TAAAGGTACC TATCTCCACT GACGTAAGGG
      TAAGAAACTG AAATTGTGAT TACAGTATAC GAAAGTTTAA TTATTAGGCT ATTTCCATGG ATAGAGGTGA CTGCATTCCC
 ----------------------------------------------------------------------------------------------

                                                                                        BamHI
                                                                                        ~~
1441  ATGACGCACA ATCCCACTAT CCTTCGCAAG ACCCTTCCTC TATATAAGGA AGTTCATTTC ATTTGGAGAG GACACGCTGG
      TACTGCGTGT TAGGGTGATA GGAAGCGTTC TGGGAAGGAG ATATATTCCT TCAAGTAAAG TAAACCTCTC CTGTGCGACC
 ----------------------------------------------------------------------------------------------

      BamH
      ~~~~
1521  ATCC   Seq. ID No. 17
      TAGG   Seq. ID No. 18
 ----------------------------------------------------------------------------------------------
```

Figure 24 . Physical Map of T-DNA Region of CaMV 35S Promoter-derived Binary Vectors Containing a BDPC



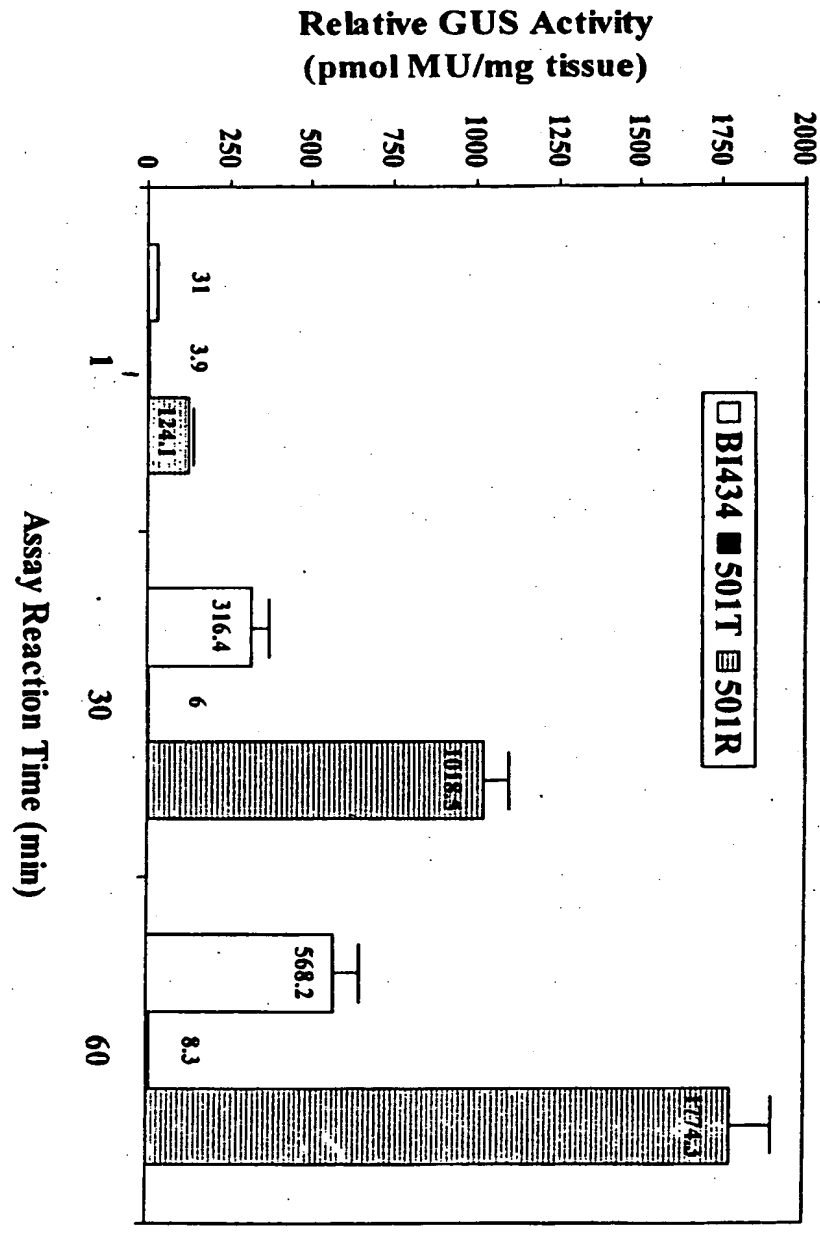


Figure 25. Analysis of GUS Activity in Grape SE (*V. vinifera cv.* Thompson Seedless) after Transformation Using Three Binary Vectors

Figure 26. Physical Map of T-DNA Region of Transformation Vectors with 4-Enhancer-Containing BDPC

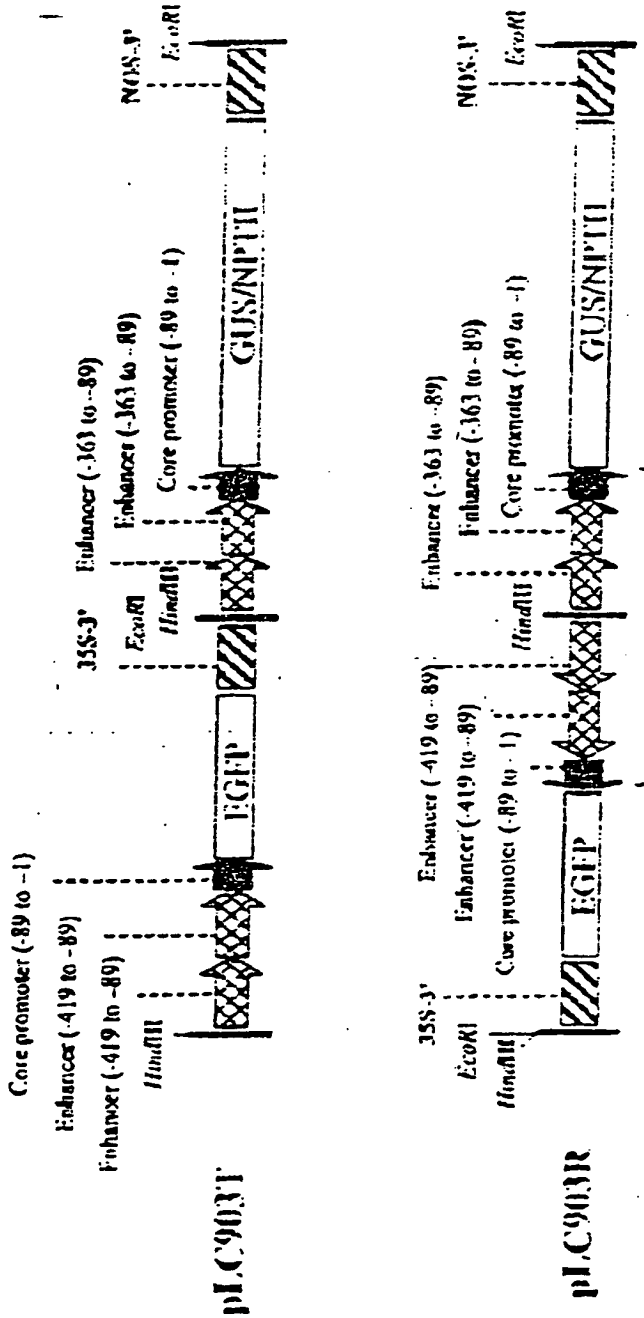
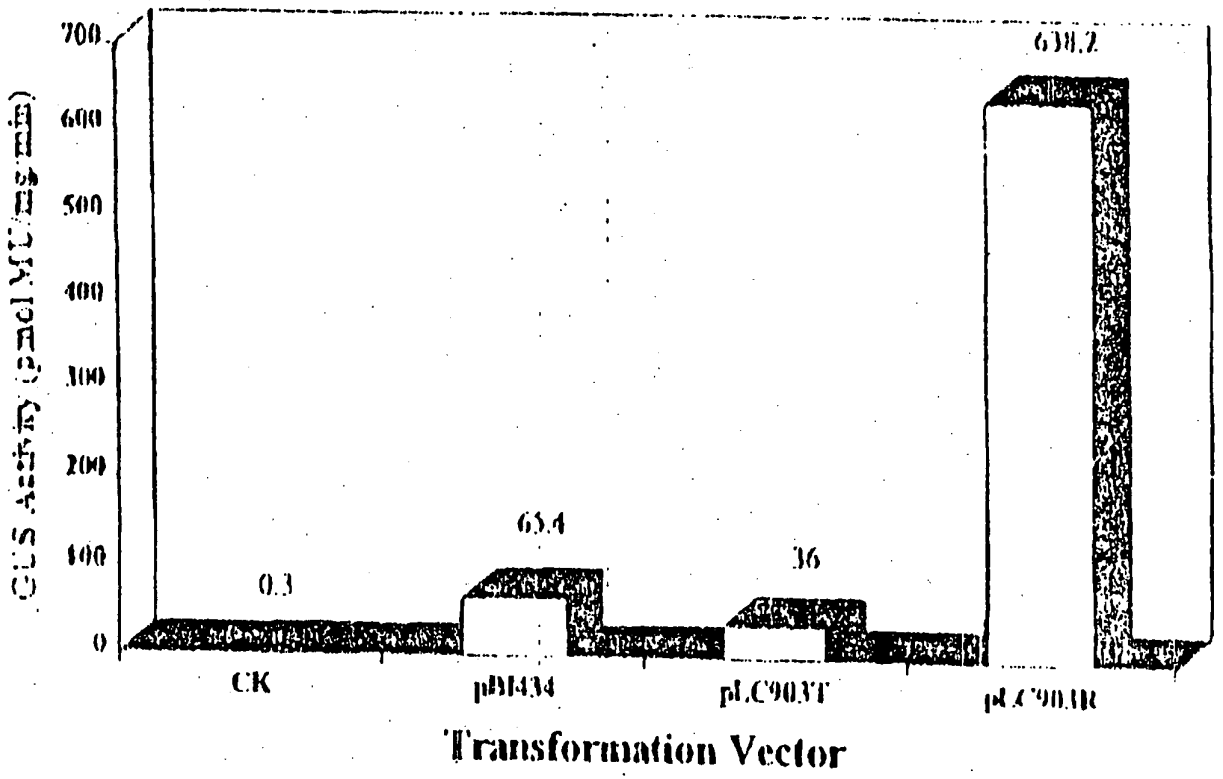


Figure 27. Analysis of GUS Activity in SE (*V. vinifera* cv. Thompson Seedless) after transformation Using Three Binary Vectors



This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.